

ACTA MEDICA SCANDINAVICA

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REDACTORES

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Studies of Erythrocyte Counting.¹

III.

Physiological Variations.

By

HENRIK F. LANGE and HERBERT PALMER.

(Submitted for publication November 21, 1947.)

In two earlier publications we have dealt with the sources of error in erythrocyte counting, especially the *purely technical errors* and what we have called *technical-physiological errors* — that is to say, all the errors due to the apparatuses employed and to the technique adopted in taking the blood samples.

In this article we will discuss a group of *variations*, which may be styled *purely physiological*, as they have no connection with the methods, but are due to various factors acting upon the particular individual.

The concentration of erythrocytes in each individual will not be found constant. It will vary according to different physiological factors, such as age, nutrition, position of the body etc. The cause of many of these variations must be sought for in varying hydration of the blood. For alterations of the blood volume will mean that the ultrafiltrate will pass in and out through the capillary membranes, while the colloid and formed constituents of the blood will be retained. The content of erythrocytes (and protein) will vary in reciprocal proportion to the blood volume. As the newformation and destruction of erythrocytes under physiological conditions will counterbalance each other, and as there will likewise be no disproportion between circulating blood and blood-

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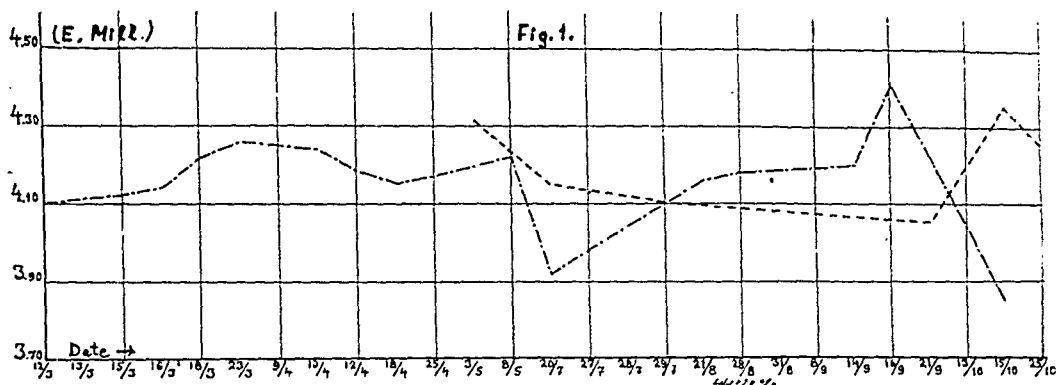


Fig. I.

Results of erythrocyte counts in capillary blood (-----) during 6 months and in venous blood (— · — · —) during 7½ months in the same individual (standard conditions).

depots, the above-mentioned changes in erythrocyte concentration must be characterized as *relative*, i. e., secondary to the alterations in blood volume. As the blood volume under basal (standard) conditions remains very constant (but shows great individual fluctuations under certain influences), the concentration of erythrocytes under basal conditions is consequently also constant (but otherwise varies greatly inversely with changes in blood volume).

In Lange's publication respecting the relative variations of plasma protein under physiological conditions it has been sought to judge of these variations on the basis of the concentration of erythrocytes. We will therefore in the present article bring forward some of the considerations which Lange in his paper advanced with respect to the plasma protein. Some of the experiments described below are referable to that essay. The fact that these experiments are based on analyses of venous blood is without significance, since, as stated in our two previous articles, we believe that with exercise of care exactly the same results can be attained in venous as in capillary blood. We will try to prove this assertion by aid of Fig. I.

Broadly speaking, the variations in blood concentration may be divided into two types: *rapid* and *slow*.

The influences which may bring about *rapid* changes are the following: Intake of liquids, work, position of body.

The influences which may lead to *slow* changes are: Height above sea-level, pregnancy, season of year, age.

The remaining factors may be disregarded in this connection, since some of them are too ill-defined to allow a discussion (cli-

STUDIES OF ERYTHROCYTE COUNTING.

mate, race, constitution), while some have been shown to have no influence under physiological conditions (for example, diurnal variation, nutrition).

In the following we shall deal with the above-mentioned factors in the order named.

1. Intake of Liquids.

It has been demonstrated by numerous authors (Marx alone mentions 24) that ingestion of liquid leads to hydration of the blood, with consequent reduction of the protein and erythrocyte concentration. In order to get a marked variation, however, abundant quantities of liquid — from $\frac{1}{2}$ to 2 litres — must be consumed in the course of a short time. In everyday erythrocyte counting this source of error may therefore be disregarded.

2. Work.

The influence of work on the erythrocyte concentration is also well known. One of the first to study this question was v. Willebrand, who in 1903 found in 12 persons an increase in the erythrocyte concentration of from 2.9 to 23.4 per cent — average 12.3 per cent — after vigorous muscular exertion. This finding has since been repeatedly confirmed by other authors. The explanation of this increase is somewhat complex. Among other things, it must be reckoned that, in addition to a change of concentration, there will also come an increase of the total number of circulating erythrocytes, owing to cells being cast into the circulation from absolute or relative blood depots. That these changes in blood concentration may be very considerable is shown by Fig. II.

The results of the experiment demonstrated in Fig. II doubtless represent extreme values, but they also go to show that immediately before the taking of blood-samples for erythrocyte counts, the patients ought to keep relatively quiet, so that the conditions may as far as possible be basal and capable of comparison.

3. Position of Body.

In experimental studies under standard conditions it has always been deemed highly important that the subject of experi-

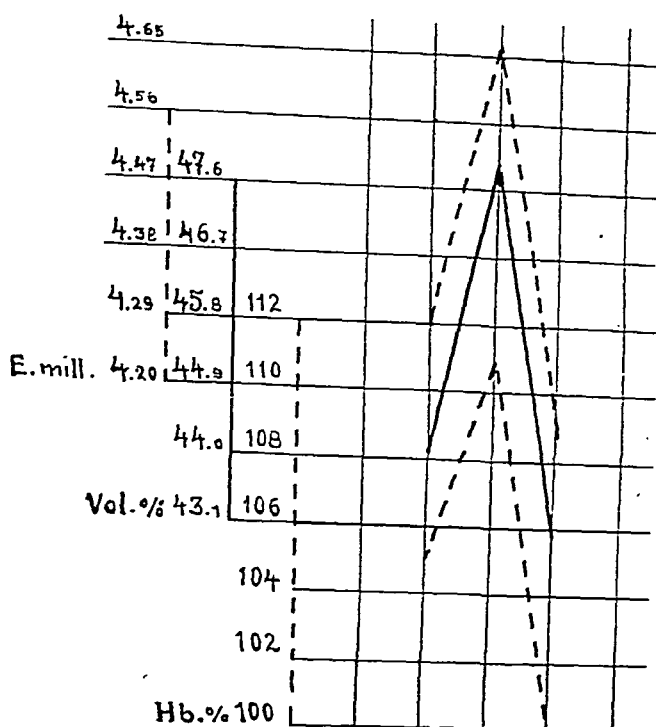


Fig. II.

Results of determination of number of erythrocytes, cell volume (vol. %) and hemoglobin concentration (Hb. %) in an individual before, immediately after and 1 hour after 8 minutes' stair-climbing exercise.

ment shall be fasting, while less significance has been assigned to his having been in a state of rest.

Meanwhile, it has never been demonstrated that intake of food produces any change in the blood concentration, so that the only reasonable explanation of the demand for a fasting state must be that it ensures that the person concerned will not have drunk any liquid. As already mentioned, however, the influence of the ordinary daily consumption of liquids is unimportant. On the other hand, the position of the body has a very great influence. In his essay, from which the following Fig. III is taken, Lange showed that the *average* difference in blood concentration between lying in bed and those who were up and going about was 8 per cent, and that there was actually found a difference of over 20 per cent in one and the same individual.

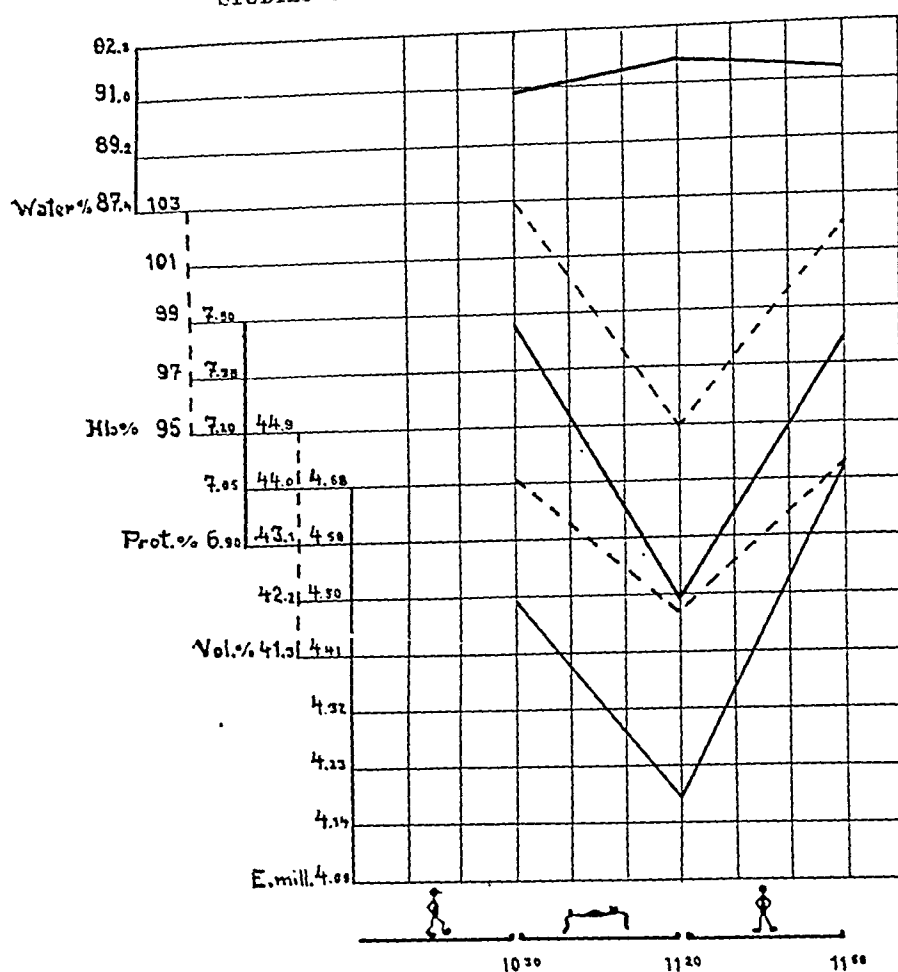


Fig. III.

Changes in concentration of plasma water (water %), of hemoglobin (Hb %), of plasma protein (prot. %), in cell volume (vol. %) and in number of erythrocytes in a person during 50 minutes' rest.

That the conditions are the same in capillary as in venous blood is shown by the following Table 1, which gives the results of three experiments we have made.

The figure and the table show that we have here a matter that is of the very greatest importance in the practical work of erythrocyte counting.

The changes manifest themselves in all the circumstances of everyday life, and it is therefore understandable that there has hitherto been no agreement respecting the *diurnal variations* of the erythrocytes, seeing that those who have made investigations in this field have paid no regard to the factor represented by the

Table 1.

Results of Erythrocyte Counts in Venous Blood and Finger-Blood Taken from Three Persons both in Standing and in Recumbent Position.

Person		E. in mill.		Vol. %	
		venous	capill.	venous	capill.
V. F.	Standing	5.01	5.12	45.4	46.2
	Lying	4.79	4.80	44.0	45.0
M. L.	Standing	3.89	3.79		
	Lying	3.55	3.29		
W. S.	Standing	4.51	4.60		
	Lying	4.49	4.42		

position of the body. Lange's investigations respecting the diurnal variations in blood concentration show the constant of erythrocytes (and protein) will be found *quite constant* the whole day round when the subjects are examined under standard conditions.

We shall now proceed to say a few words about the factors which produce less rapid changes in the number of erythrocytes than those mentioned above do. It is here a question of changes extending over *somewhat longer periods*.

1. Height Above Sea-Level.

The physiological polyglobulia occurring at high altitudes has been known to physiologists for many years. It has been especially studied by Barcroft during an expedition to the Andes in 1926—27. He found that persons living at an altitude of 4,000 to 5,000 m over sea-level had an erythrocyte concentration which was 43 per cent above the normal average (with range of variation from 24 to 69 per cent). This increase is real and has nothing to do with the change in blood volume. It will always take some time, however, before an individual's erythrocyte content adapts itself to the reduced oxygen tension.

2. Pregnancy.

Pregnancy offers one of the most striking examples of great changes in concentration owing to hydremia. This hydremia in-

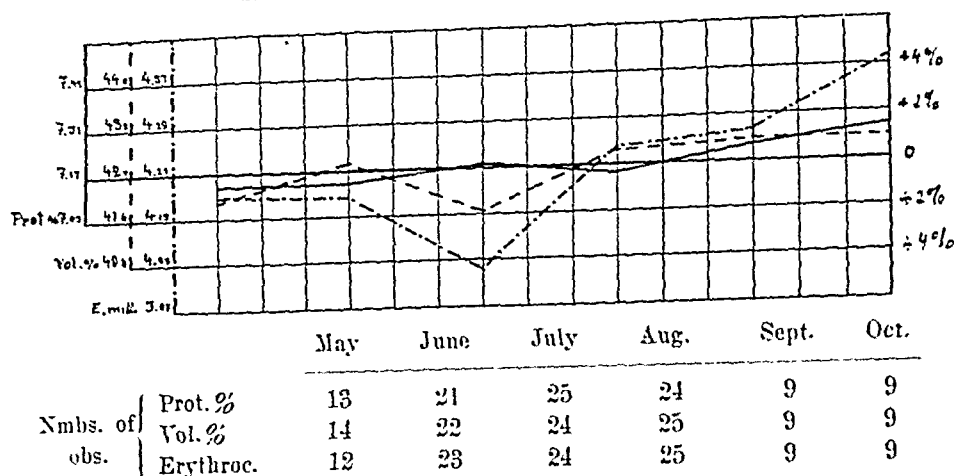


Fig. IV.

Average results of numerous observations of plasma protein concentration (prot. %), cell volume (vol. %) and number of erythrocytes in 9 normal subjects of experiment in the course of half a year (Oslo, 1943).

creases in the course of the pregnancy, and it increases on occurrence of toxicosis, during which the hydremia and the so-called »physiological anemia» may reach to considerable degrees of severity.

3. Season of the Year.

This concept is not clearly defined and it includes a large number of factors, the most important of which is presumably represented by variations in temperature. Hereto comes in the northern lands the irradiation of light. On account of the complexity of the active factors it is difficult to judge whether the variations in erythrocyte content are relative or absolute. The findings in Lange's investigations (see Fig. IV) go to indicate that the variations are of absolute nature.

The changes are so slight, however, that they need hardly be taken into account during ordinary work.

4. Age.

During the development of the individual there occur changes in the concentration of erythrocytes. For instance, it has been clearly shown by Williamson that the polyglobulia of the newborn child is succeeded by the relative anemia of the nursing infant and that the values then rise again until the age of puberty.

Likewise these variations play no rôle in the ordinary work of investigation, and they are mentioned here only in order to give a more complete survey of the matter.

To sum the matter up, we must point out that in the counting of erythrocytes — whether for the purpose of establishing normal values or when making serial investigations — due regard must be paid to those factors which bring about changes in the individual's erythrocyte concentration.

We must — in order to obtain comparable values — always take care to work under standard conditions. In order that the results attained by one investigator or in one laboratory may be comparable with those reported by others a description of these condition must always be furnished.

Summary.

In this article the authors deal with the factors that may produce a change in the concentration of erythrocytes. Through personal investigations they have shown that the following points are of practical importance: For rapid changes: Copious intake of liquids, work, position of body. For slow changes: Pregnancy, season of year, (height over sea-level), (age).

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Paroxysmal Ventricular Fibrillation with Recovery.

By

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(Submitted for publication November 24, 1947.)

Ventricular fibrillation was first observed to occur immediately before death (Robinson 1912, Halsey 1915); and there is reason to suppose that ventricular fibrillation very often occurs at the moment of death.

Paroxysmal, ventricular fibrillation or flutter with survival has so far been regarded as an extremely rare phenomenon. A number of cases (ab. 25) have, however, been reported of late years (1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 16, 17, 18), cases which have been verified by electrocardiography. By the nature of the case electrocardiograms are often difficult to obtain during the rather brief fits. It is, therefore, a question whether this phenomenon is not more frequent than it is generally supposed to be, also as the cause of Adams-Stokes' syndrome.

A review of the literature in hand shows us that nearly all the observed cases of ventricular fibrillation were preceded by a severe heart disease, often with attending dissociation between auricles and ventricles, and all the patients except 3 (1, 5 and 7) died shortly after the ascertainment of the ventricular fibrillation. Complete restoration of the heart occurred in only 2 of the reported cases (1 and 5). In these cases the patient remained well and able to work. A similar case, which will be described below, has been observed.

A detailed account of the pathogenesis in ventricular fibrillation and of Adams-Stokes' syndrome can be omitted here, since these

facts have been dealt with at large by other writers (Spühler, Kerr & Bender, and others). Only there is reason to call to mind the fact that flutter and fibrillation in auricles or ventricles are generally supposed to arise by the normal impulses being replaced by circular movements in the muscles of auricles and ventricles respectively.

There still seems to be some uncertainty with regard to the nomenclature and the distinction between ventricular tachycardia, ventricular flutter, and ventricular fibrillation. In the cases of ventricular fibrillation quoted from literature the rate is generally rather low — in some of Kerr & Bender's tracings as low as 120, and in many cases between 200 and 300 (2, 5, 6, 7, 13, 16, 18). This nomenclature accords badly with that applied where auricular fibrillation is concerned. Here we speak of fibrillation only when the rate exceeds 400. Boyd & Scherff hold the same view, since, to make a diagnosis of ventricular fibrillation, they require irregularly shaped F waves in very rapid and irregular succession (rate ab. 500).

Katz holds that ventricular flutter should be diagnosed in the electrocardiogram when *regular* continuous waves of large amplitude occur at a rate over 250 per minute and ventricular fibrillation by the absence of QRST complexes and the presence of *irregular* undulations of varying amplitude, contour and spacing. The rates of these vary from 250 to 500 per minute. The waves are larger than in auricular fibrillation and no ordinary complexes are seen.

The latter nomenclature will be used in this paper.

Case history: Case 771/46. Man, aged 50. Was in hospital from Febr. 28 to March 2 and again from March 6 to April 27, 1946. Rheumatic fever with signs of cardiac affection at the age of 28. Has since at periods had pains in his joints. Within the last few years a few fainting fits, the last fit about 6 months ago. Has, moreover, been suffering a great deal from palpitation and from fits of dyspnoea at night. Can work, ride on bicycle, and walk up stairs without trouble. Three weeks before his first admission to hospital the patient observed a long tapeworm in the feces. Was treated with extract of fern, 10 g, after which a tenia with scolex was discharged. No cardiac complaints. Was re-admitted three days after his discharge. The day after his return home he had felt unwell with nausea, vomiting, and diarrhoea. On the day of re-admission the patient had several attacks of pains in the heart attended with sensation of fear, as well as fainting fits. Within the following 24 hours the patient lost consciousness several times, about 4 times per hour. The fits were not preceded by symptoms of

any kind, but came on suddenly with indisposition increasing to unconsciousness. The patient became cyanotic, there occurred minor convulsions, and the respiration increased violently in strength. As a rule the patient discharged urine and feces. He recovered surprisingly quickly after the fits. Between the fits the pulse was quite regular with a rate of between 80 and 120. At the onset of each fit the pulse disappeared suddenly without preceding irregularity. By the end of the fit the pulse returned, irregular at first, but in the course of a few minutes it became quite regular. No heart action was heard during the fits. After the fits had been shown through electrocardiography to be fits of ventricular fibrillation the patient was given sol. g.-strophantin, $\frac{1}{4}$ mg i. v., on March 7 at 7 p. m. and again on March 8 at 1 a. m. He then slept quietly through the night and had no fits till the next morning, when he had three fits exactly like those described above at intervals of one hour. An electrocardiogram was registered during the entire period of the last fit. The patient was now given strophantin, $\frac{1}{4}$ mg \times 2 at an interval of 4 hours, and at the same time a digitalis treatment was commenced: 60 cg falling in the course of 2 or 3 weeks to 10 cg daily. On March 17 the pulse rate was 60 and the pulse was regular. No fits occurred after the commencement of the digitalis treatment, and, apart from an occasional slight feeling of trouble at the heart, the patient felt perfectly well during his entire stay in hospital. From March 26 to April 8 the patient was given 5 cg fol. digitalis, after which the digitalis treatment was discontinued. The rest of the time in hospital the patient's heart action was regular, ab. 80, and there was no tendency to any fit. The patient has been feeling perfectly well since his discharge from hospital twelve months ago and is fully capable of working.

Physical examination (out of fits): Height 161 cm, weight $47\frac{1}{2}$ kg. Somewhat pale and slender. No cyanosis nor dyspnoea. Eyes normal. Pupillary reflexes normal. No reddening in the fauces. Steth. of lungs: normal conditions. Steth. of heart: Action regular with no pulse deficit. Abdomen normal. Extremities normal. Reflexes normal.

Examinations: Hg. 100 %. Sedimentation rate 6—12—9 mm. Urine: no alb., sugar, pus, nor blood. W. r. negative. X-ray of the lungs on March 16, 1946 revealed distinctly marked fibrous band-like changes stretching from the right apex down towards the hilus. There were no signs of decay and the changes did not seem to be quite fresh. The remaining parts of the lungs presented no signs of infiltrative or pleural processes. Shadows of cardiac vessels normal. X-ray examination of the heart by ortodiagraphy on April 3 showed the shape and size of the heart to be normal. X-ray of the heart on Oct. 10 and April 1947 showed the heart to be normal of shape and size.

Electrocardiograms. An electrocardiogram registered five days before the fits revealed nothing definitely abnormal. P—Q extended over 0.20 sec.; there was slight splitting of R_3 , and T_3 was negative (Fig. 1). Electrocardiograms registered during Adams-Stokes' fits (Figs. 2, 3, 4) revealed ventricular fibrillation or flutter at rates of 300 to 360. The tracing is seen as an almost regular wave line in all three leads, with no

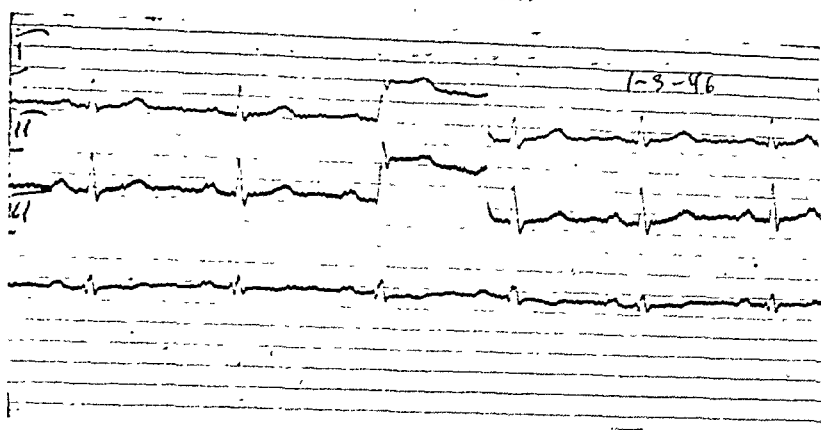


Fig. 1. Electrocardiogram 5 days before fit of ventricular fibrillation.

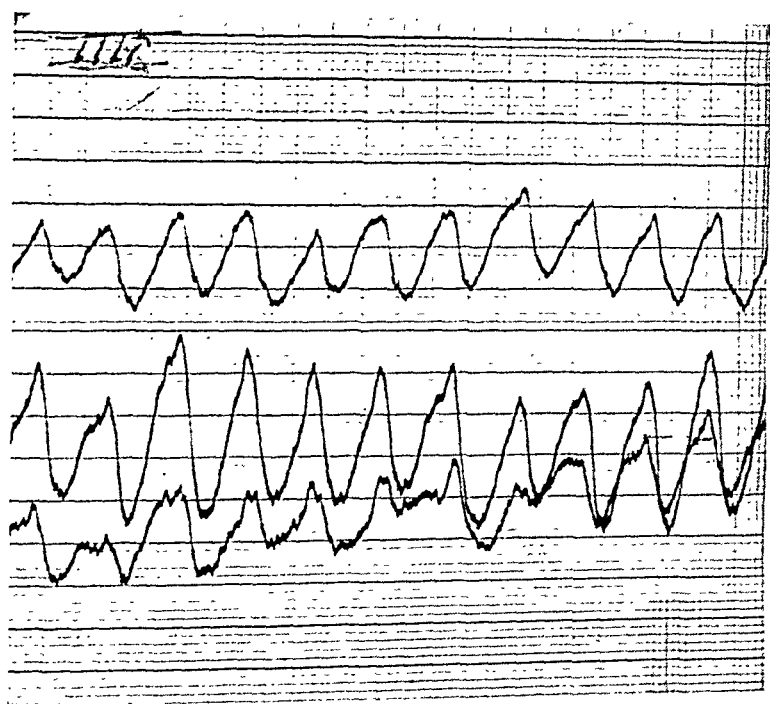


Fig. 2. Ventricular fibrillation. Rate 300.

iso-electric line between the ventricular complexes. The individual complexes are not quite uniform. An electrocardiogram was registered during the entire period of one of the fits. There was found to be ventricular fibrillation or flutter for 125 sec. (Fig. 4.) After a ventricular pause of 1.4 sec. there followed a low, broad ventricular complex (Fig. 5). Next there followed at long intervals (up to 3.5 sec.) ventricular com-

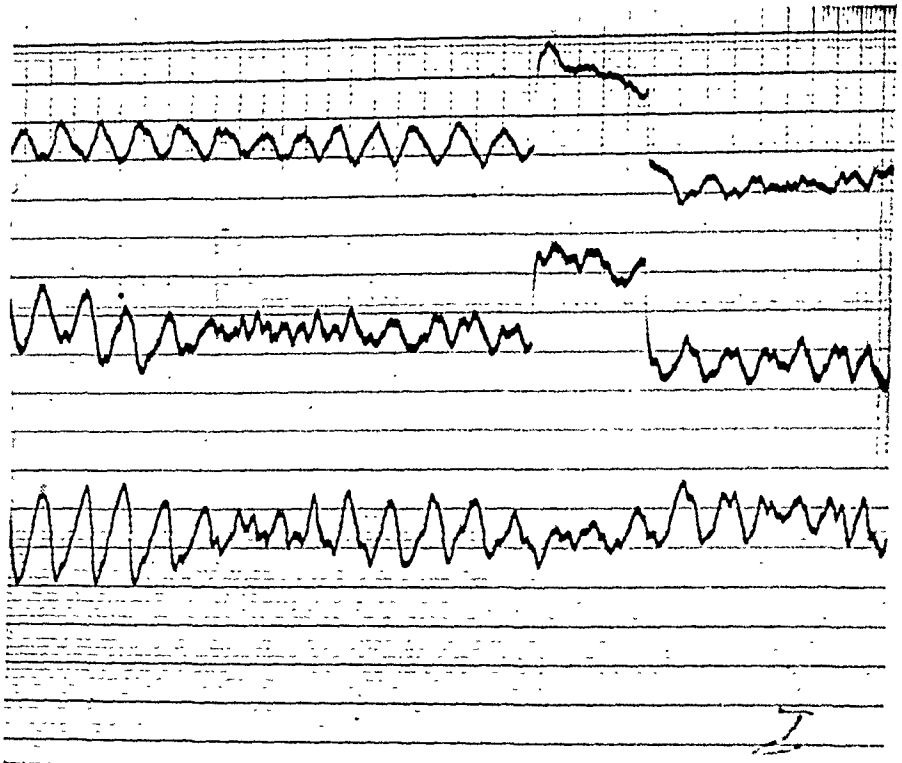


Fig. 3. Ventricular fibrillation. Rate 360.

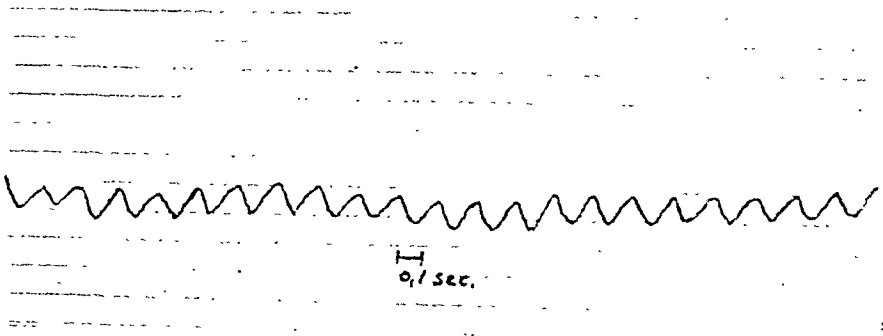


Fig. 4. Ventricular flutter 35 sec. after onset of fit. The electrocardiograms fig. 4-7 have been made with reduced sensibility.

plexes of varying and irregular shapes (Fig. 6). Simultaneously there occurred P waves at regular intervals of 0.6 sec. There was complete dissociation between auricles and ventricles. After a period with ventricular extrasystoles differing in shape and rate there followed one of regular ventricular complexes without permanent connection with the auricles (Fig. 7). Finally, after a period with a much prolonged conduc-

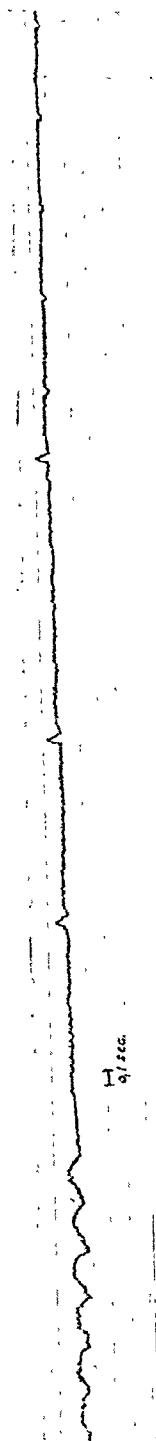


Fig. 5. Ventricular flutter 125 sec. after onset of fit with transition to ventricular rhythm with dissociation between auricles and ventricles.

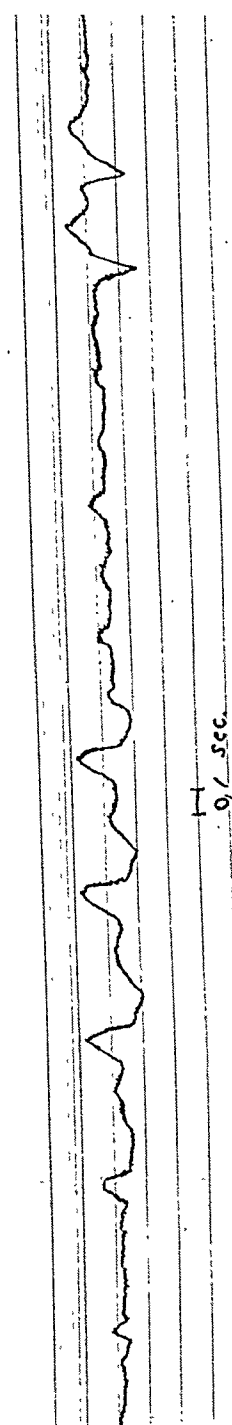


Fig. 6. Ventricular complexes of varying and irregular shapes 155 sec. after onset of fit.

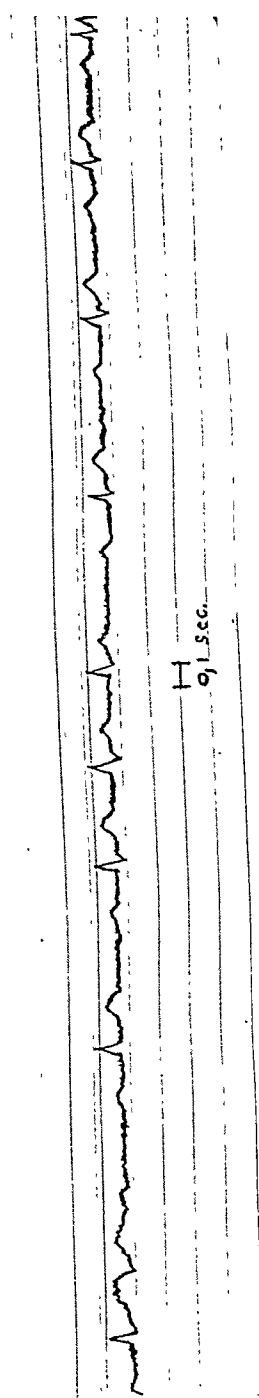


Fig. 7. Auriculo-ventricular dissociation 255 sec. after onset of fit.

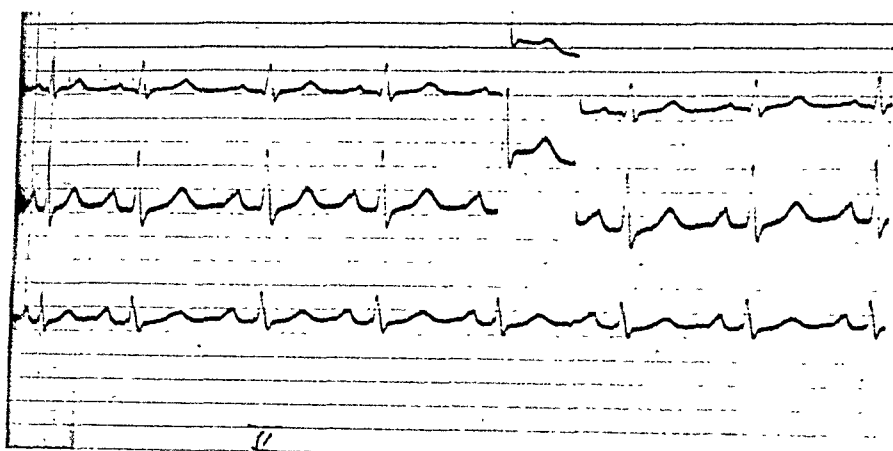


Fig. 8. Normal electrocardiogram 3-4 min. after fit.

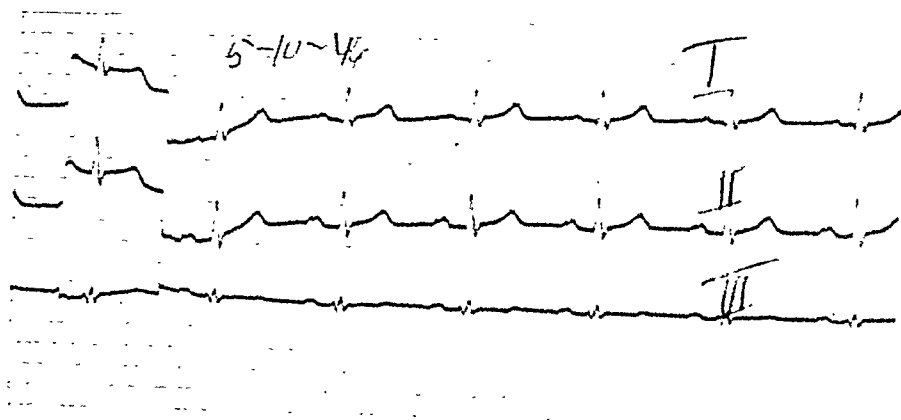


Fig. 9. Normal electrocardiogram 6 months after last fit.

tion time and occasionally dissociation, the heart passed into a normal sinus rhythm.

A perfectly normal electrocardiogram (Fig. 8) could be registered from 3 to 6 minutes after a fit. We did not succeed in demonstrating anything abnormal in heart rhythm or electrocardiogram immediately before a fit.

During the digitalis treatment there was once found a prolonged conduction time (March 19: P—Q 0.26 sec.). In none of the remaining electrocardiograms P—Q extended over 0.20 sec., and there were found no other electrocardiographic changes of any note. Was discharged with a normal electrocardiogram. An electrocardiogram registered six months after the discharge of the patient revealed nothing abnormal (Fig. 9).

Subsequent course: One year after the patient's discharge from hospital he was demonstrated in *Dansk Selskab for Intern Medicin* (the Da-

nish Society for Internal Medicine). The patient had since his discharge been feeling well and was fully fit for his work. He had possibly had a minor attack, since he stated that one day he suddenly lost what he had in his hands. He did not fall. On April 13, 1947 the patient suddenly dropped dead without preceding malaise.

Postmortem examination was undertaken in the Medico-Legal Institute of the University. The skull was opened. Nothing abnormal was found in brain and meninges.

The heart measured $9\frac{1}{2}$ cm in length and $10\frac{1}{2}$ cm in breadth. It weighed 330 g (normal weight ab. 270 g), being thus slightly enlarged. Valves and ostia, as well as endo- and pericardium presented normal conditions with no signs of present or past inflammation. To the naked eye the myocardium was likewise normal, of a fresh red colour and with no signs of fibrosis or myomalacia. Hemorrhages were not observed either, more particularly not over the area of the conduction bundle. There were no signs of deformation, the foramen ovale was closed. Coronary arteries: The ostia were of normal width. In the first part of the left coronary artery, extending ab. 4 cm down through the descending branch, there were seen some yellowish, pad-like thickenings of the intima, but without macroscopic sclerosis or necrosis. The lumen was normal, or approximally normal in width. There were seen no thrombi nor emboli nor remains of such. Slight, yellowish, pad-like thickening of the intima was seen in the first part of the circumflex branch, but otherwise nothing abnormal. The same was the case in the right coronary artery. The aorta was of normal caliber. There were found scattered, yellowish, pad-like thickenings of the intima, notably in the descending branch.

Stomach, intestine, liver, bile ducts, suprarenal glands, kidneys, urinary tract, and spleen were normal.

Histological examination of the heart: A section was taken of the myocardium from both ventricles, and from the superior part of the septum. The myocardium was seen under the microscope to be well-preserved with rather little fragmentation. The muscular fibrils were of normal appearance with preserved cross-striation. There was moderate hyperemia of the vessels. Interstitial inflammatory processes or fibrosis were found now here. The endocardium was normal throughout. The fatty tissue in the epicardium was of normal appearance. In some very few places there were seen a few lymphocytes round some small vessels. One of the sections showed a coronary branch, which presented atheromatosis, thickening of the intima with hyalination, and a few spaces having contained lipid. There was no sclerosis.

All in all there were demonstrated no pathological changes except for slight atheromatosis of the coronary vessels.

Discussion.

To my knowledge there is found described in literature no more than one case (5) of paroxysmal ventricular fibrillation with Adams-Stokes' syndrome, where this fibrillation has developed

without preceding signs or symptoms of a myocardiac affection, and where the patient seemed to recover completely.

The question now suggests itself whether the heart disease had a special, possibly toxic, cause in these two cases. The patient had shortly before been under treatment for tapeworm with extract of fern. The pharmacological text-book literature gives only very little information on the toxic effect of this drug on the heart. Goodman & Gilman mention, however, cardiac failure as a toxic symptom, and Poulson recommends cautiousness with the treatment in the cases of heart diseases. Espersen mentions in a report of 191 tapeworm treatments that four patients developed tachycardia and one got Adams-Stokes-like fits. No cases are reported of development of ventricular fibrillation. The present patient, however, had two fainting fits 12 to 6 months before the treatment and died probably in a similar fit 14 months after treatment. These facts go against regarding the fits of ventricular fibrillation to have an exclusively toxic cause. The causative relation is thus difficult to make out. The rheumatic cardiac affection developed in connection with rheumatic fever and the fainting fits mentioned above argue in favour of an already existing heart disease, even though a normal electrocardiogram was registered on the patient's first admission, and the heart has never been found to be abnormal in shape or size.

The extract of fern may, perhaps, here have brought about ventricular fibrillation in a heart which was predisposed to this anomaly. The patient had been given neither digitalis, strophanthin, nor quinidine sulphate, which are known sometimes to cause ventricular fibrillation (Kerr & Bender, Zwillinger).

Ventricular fibrillation may likewise be brought about by electric shock, coronary occlusion or other causes of severe anoxemia, trauma of heart or wall of chest, as well as chloroform or cyclopropane anesthesia (1).

It was striking to see how well the patient felt between the fits and how soon he recovered after the both severe and rather protracted Adams-Stokes' fits. The explanation is no doubt to be sought in the fact that the heart action was comparatively normal (ecg. normal) between the fits. In most of the cases described of paroxysmal, ventricular fibrillation the fits were followed by an irregular heart action with states of weakness or semi-coma persisting for several hours.

The patient died quite suddenly thirteen months later. The

postmortem examination revealed nothing abnormal, neither macroscopically nor microscopically. Presumably he died in a fit of ventricular fibrillation; but actually we have no proof that he died from a cardiac disease at all.

This indicates the interesting fact that ventricular fibrillation may develop in a heart where no damage can be demonstrated, neither through electrocardiography, nor by naked-eye or histological examinations.

It is impossible, on the basis of the literature in hand, to indicate definite lines to be followed in the treatment of ventricular fibrillation or flutter. The experiences made by the individual writers are very limited, and the underlying heart diseases are different.

In our case strophantin given intravenously with ensuing digitalis treatment proved to have a surprisingly prompt and lasting effect. Zwillinger repeatedly succeeded in removing fibrillation by intracardial injection of 10 ml of 15 % magnesium sulphate. In a case in which there was finally complete arrest of the ventricular action Levine & Matton observed contractions of the ventricles immediately after injection of adrenalin intracardially. Euphylline and theophylline as well as atropine are recommended in the cases in which there is coronary sclerosis. Quinine and quinidine are recommended as means to increase the refractory period and to slow the impulses in the cardiac muscles. Dock kept a patient free from fits for one year by means of quinidine.

The differential diagnosis against auriculo-ventricular block, the affection which most frequently brings about Adams-Stokes' syndrome, is of importance. In the case of auriculo-ventricular block the syncopes occur most often at changes from sinus rhythm to ventricular automatism. Accordingly a pulse rate changing from 80 to 40 is indicative of this disease. However, it should be borne in mind that ventricular fibrillation frequently occurs in connection with dissociation between auricles and ventricles. The proof of the presence of ventricular fibrillation or flutter can be given only by electrocardiography.

Summary.

A case is reported with numerous fits of paroxysmal ventricular fibrillation or flutter (rate 300 to 360) attended by Adams-Stokes' syndrome. The disease came on suddenly after a treatment for tapeworm with 10 g extract of fern, and disappeared after administration of strophantin, $\frac{1}{4}$ mg i. v., followed by a

digitalis treatment. The patient felt perfectly well 13 months later with no signs or symptoms indicative of a heart disease. He then died suddenly. On autopsy a heart disease could not be demonstrated neither macroscopically nor microscopically.

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Libman-Sack's Syndrome.

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The somewhat variable and etiologically still obscure symptom complex best known under the term »Libman-Sack's» disease is gradually being viewed from different standpoints.

In an exhaustive report published in 1939 by Reifenstein (Arch. Int. Med. 63. 1939) the syndrome is described as a »variable symptom complex of undetermined etiology with fatal termination», and in 1943 Cai Holten defined it in Hospitalstidende as a »malignant polysero-synovitis with cutaneous manifestations» and discusses the cutaneous changes earlier described as lupus erythematosus disseminatus.

The author has had the opportunity of following such a case with a relatively acute course, which will now be portrayed.

Miss K. T., 45 yrs. (Journal Nr. 447/1947) visited the author at the end of April suspecting that she was suffering from rheumatoid arthritis presumably contracted in her cold, damp house during the exceptionally severe winter. She had noticed the first signs of her trouble during the latter part of December, 1946. In the anamnesis was to be read: 1927 right tb-coxitis for which pat. was nursed at a seaside sanatorium; 1933 tb-osteitis in one rib for which pat. was again sent to a sanatorium; 1935 nephrectomy for stones in the kidney; 1941 thyroidectomy on account of Grave's disease. The last five years she had felt fit and had earned her living as a dressmaker.

The *subjective* troubles of her present disease began at Christmas time, when she noticed insidious pains in the joints and in various muscles. She felt no severe pains but rather a stiffness in the right shoulder-joint, the left knee-joint and in both wrists. After a few weeks' trouble the patient had high fever and more pains, which she interpreted as influenza, in spite of the fact that she had no

LIBMAN-SACK'S SYNDROME.

symptoms in the upper airways. The high temperature (approx: 39°) lasted about a week, after which the pains in the joints and muscles became more troublesome. Pains and a slight swelling attended by a feeling of stiffness now occurred also in the phalangeal joints of the fingers and in the joints of the big toe, and the patient became sub-febrile.

For about two years the patient had noticed a purple spot about the size of a pea on the dorsal side of the proximal phalanx of the right index finger. During the last few months this spot had shown a tendency to grow, but gave the patient no trouble. In the severe winter of 1946—47 she had noticed a cold feeling in her fingers which easily became cyanotic and at the tips she observed the appearance of purple spots. At the same time she noticed a bluish discoloration of the tip of her nose where the skin felt dry and now and then peeled slightly. The last week the inferior portion of her right calf had become tender and painful but she had not noticed any swelling. She took salicylica for her pains, but in vain. Up to 1945 she had been under regular control of the tuberculosis dispensary on account of the tuberculous diseases she had had.

When the author first examined her on 21st April, 1947, her condition of health was good. She was somewhat thin and had ankylosis of the right hip-joint but she could walk without trouble. The right knee-joint was slightly swollen and was 90° flexible. No palpation tenderness over the joints. The joint symptoms were thus objectively very sparse. The changes in the patient's skin, however, were astonishing, especially acrocyanosis most conspicuous in the fingers and nose. On the volar side of the distal phalanges of the fingers were a number of slightly projecting, purple, well defined spots, varying in size between that of confetti and that of a pea, which were and there showed a tendency to peel peripherally. Cyanosis extended, vague and butterfly-like, over her cheeks. On the inferior surface of her toes were a few scattered but less conspicuous spots similar to those on her fingers. The patient had a somewhat tender hazel-nut sized lymphadenitis on her neck immediately below the right jaw bone. In the right calf a rather superficial, somewhat tender thrombophlebitis, about 3 cms long, was palpated. The clinical examination of the internal organs revealed nothing remarkable. Pat. was afebrile. S. R. gave an unexpectedly high value, 120 mm/p. hour, for which reason the complexus was deemed so obscure that she was admitted to Vasa Hospital, Gothenburg, for observation. The S. R. had also been tested in a refrigerator, a thing we often do with unclear cases of max. S. R. values, and it was found to be 31 mm p. h.

In view of the fact that a difference was found at the first examination between the S. R. at room temperature and in the refrigerator, the examination was repeated, besides which the sero-albumen was examined. Formol-gel test was positive. It was then discovered that it was a question of hyperglobulinemia. (See Table.) Continued examinations revealed slight anemia with an index of approx. 1 and

leucocytosis with a »shift to the left». Examination of the bone marrow divulged nothing of interest. Thrombocyte values were normal, as was also the case with the iron content of the blood-serum. In order to exclude myeloma (hyperglobulinemia and diffuse pains) and tuberculosis (Joint symptoms characteristic of Poncet's disease) both the skeleton and the lungs were X-ray examined, but no positive findings could be detected. The E. C. G. was normal. A dermatologist (J. Fex) was consulted who reported that the cutaneous changes were presumptive of lupus erythematoses.

Except for a very transient inexplicable fever (over 40°) a few days after hospitalization the patient was at first sub-febrile but recovered quickly, and at the end of May, after a few weeks' stay at the hospital, she was afebrile and but for certain stiffness of the wrists was free of subjective pains and therefore released for further observation. The S. R. was still high but leucocytosis had disappeared. The objective finding was otherwise unchanged. During a few days towards the end of this stay at the hospital, however, there appeared a number of petechias quite unexpectedly along both shins. Thrombocyte and ascorbin values were normal then as was also the prothrombin value. Rumpel-Leedes test was negative. The cutaneous changes had moreover now gradually receded and had by this time, the end of May, practically disappeared. The only thing left being the faded efflorescence on the right index finger and very slight acrocyanosis (Lab. results are shown in the Table 1).

After being at home six weeks the patient was re-hospitalized on 3rd July, 1947. The first few weeks she was afebrile but then became subfebrile. Aches, pains and a feeling of stiffness in the joints, and in the musculature of the stout joints, reappeared. She began to feel tired and suffered from less of appetite, but she was not short of breath, neither did she complain of any pains in the chest. No abdominal symptoms. The patient had avoided sun bathing but nevertheless her face became redder and redder.

On renewed examination the bright red colour of the patient's face was astonishing as was also a slight edema around the eyes. On the cheeks and around the lips the skin was somewhat scaly. The skin of the inferior part of the neck and above the jugular region was cynaotically red and displayed several scattered cyanotic papules the size of a pea and smaller, and pale patches. There was a sharp demarcation between these phenomena and the normal skin, which gave the picture a more erysipelatous appearance than ever. (Fig. 1.) The patient felt no itching but an uncomfortable burning in the face. A clinical examination of the internal organs and the joints disclosed no new, additional changes. Bilaterally and distally of the calves were some subcutaneous cords, a few centimeters long, which were tender and interpreted as phlebitis. An examination of the blood still showed a high S. R. of the same type as before as well as hyperglobulinemia. The slight anemia had not progressed. Her temperature remained a little over 38°. A piece of skin for histological examination was taken from the jugular region and from the suspected phlebitis in the calf.



Fig. 1. The lesions of skin of face and jugular region.
(Typical for the acute "disseminated lupus erythematosus".)

After a week the patient had high fever and felt more pains and aches. The objective joint symptoms were still very sparse. The cutaneous changes in the face went on increasing, the redness of the face becoming more intense than ever, and reminded one very strongly of a morbillous exanthema. Rhagades appeared in the corners of her mouth but no enanthema. The spots on her fingers then became more cyanotic, they assumed the size of peas and were somewhat scaly though still well-defined. A few days the patient had very high fever attended by headache and dimmed vision, for which reason she was *ophthalmologically examined* whereby slight papillary edema was detected and a few small perivascular nests were suspected. An ophthalmologist (Dr. B. Ahrnberg) was consulted. He discovered small exudative flocks bilaterally adjacent the retinal vessels. These flocks disappeared after a few days only to be substituted by more pronounced edema and exudative patches. In the anterior chamber very minute opacities were discerned.

During the following few weeks the patient's condition gradually grew worse, but it was not until the last week of July that signs of

polyserositis were discernible, first left pleuritis and then ascites. Simultaneous signs of nephritis and slight increase of the non-protein nitrogen in the blood and enlargement of the liver. The patient's strength failed more and more whilst the high fever continued resistant to penicillin in large doses, and on 15th Aug. exitus occurred.

I would now mention the results of a few laboratory examinations. Vidal's test and blood cultivation turned out to be neg. even as late as the end of July, that is, when the patient had been febrile for some weeks. On examination with Wassermann's reaction in serum the latter showed anti-complementary reaction in repeated tests. Both bacteriological examination of the pleural exudate and guinea-pig tests were negative. The albumen-globulin quotient in the pleural exudate was 1 : 2. Cholesterol value of the blood was 215 mg per cent.

A closer analysis of the albuminal fraction was kindly made by Docent B. Olhagen (Carolinian Hospital, Stockholm) whose report runs as follows:

»Result of Electroforesis-Analysis of the Serum.

	Albumen	Globulin α	β	X	γ
Relative per cent.	39.1	9	12.3	26.1	13.5
Total albumen 7.1 per cent in serum.					

The serum thus contains a relatively large quantity of pathologic globulins, essentially of γ type (migrate somewhat quicker than the normal γ globulin). It does not belong to the anti-complementary globulins when the serum does not show any anti-complementary reaction in a hemolytic system after inactivation; on the other hand there was complement fixation with two extracts in Wasserman's reaction (probably non-specific phenomena).»

Concerning the ophthalmoscopic finds Dr. Ahrnberg writes: »In this case the changes in the fundus are restricted altogether to the retina and consist of small edematous and exudative zones grouped immediately around the retinal vessels, especially in the vicinity of the papilla. The individual nests have been very transient indeed but have been substituted by new ones. The vessels themselves do not exhibit any substantial changes. No great damage of the fundus. The picture coincides very well indeed with the fundus finding seen in the earliest stage and described by Bo Andersson and A. Samuelson (*Acta Med. Scand.* 1944). In both these cases one has quite naturally visualized the possibility

of some injurious agent that has been secreted by the vessels and given rise to reactive retinal reactions.»

The histological examination of the excised fragment of skin from the sternal region showed vasculitis in a number of cells with an environmental infiltration of leucocytes and lymphocytes (Dr. S. Ramström, Uppsala).

The most important changes in the blood will be apparent from the following table.

Table.

Day	S. R. 1 hour		Sero-albumen p. c.				Hgb p. c.	Red blood corp. mill.	Index.	White blood corp.
	Room t.	Refrig.	Total	Alb.	Glob.	Quoti- ent.				
21.4	120	31					76	3.8	1	8 000
28.4	119	90	7.5	3.0	4.5	0.66	72	3.6	1	10 500
20.5	122	55	7.7	4.0	3.7	1.08	67	3.4	0.98	3 600
4.7	125	10					67	3.3	1.01	6 500
17.7			8.1	3.4	4.7	0.72	63	2.8	1.12	8 500
2.8	138	70	7.2	2.5	4.7	0.53	66	3.2	1.03	8 000

Autopsy was performed 14 hours after death and revealed inter alia (only positive finds will be given): On the inner surface of the lower leg was a small fistulizing cavital abscess and thrombophlebitis (at the site of the excision of the fragment of skin). In the pericardial sac was 400 ml of clear yellow serous liquid. Macroscopically, the lungs displayed nothing remarkable except for slight left atelectasis. The liver was enlarged and swollen, it was yellowish brown in colour and its sectional surface was of normal design but showed a conspicuously fattened parenchyma. In the abdominal cavity there was 100 ml. clear yellow serous fluid. The spleen was normally sized, firm and fibrotic with a dark brownish-red sectional surface. The left kidney (r. already removed) was somewhat enlarged and had a rather loose capsule and smooth surface. The sectional surface exhibited a swelling cortex of normal breadth, well defined stroma and yellowish-brown parenchyma.

The *histological examination* produced normal pictures of the brain, meninges and basal cerebral vessels. There was a slight increase in the amount of connective tissue of the myocardium. The liver showed a conspicuous, diffuse fattening and an increased quantity of connective tissue in which moderate cellular infiltra-

tion was observable. The spleen had an abnormally high amount of connective tissue and some obliterated arteriolar and venular. The pancreas was slightly cirrhotic.

In the hilus and abdominal lymph glands a number of characteristic changes were discernible. The glands were edematous and hyperplastic with lymphocytes and plasma cells in the sinus. Most of the follicles had been obliterated by a number of necrotic patches. Arteriolar-sized vessels showed here and there obliterated lumina with thrombi and fibrinoid degeneration. Further there were a few single large cells with homogeneous cytoplasm and large, lobed nuclei (of Fox-Rosahn type). The pleura was normal but in the underlying tissue there were a few migrating lymphocytes and a few thrombosing vessels of the size of an arteriolar and smaller. In the lung were also a number of thrombosing finer vessels and a number of vessels with thrombus-like deposits. Besides this, slight emphysema and small zones of bronchopneumonia. Around the vessels and in the rest of the tissue, cellular infiltration. The kidneys exhibited fibrotic and hyaline glomeruli, as well as large swollen glomeruli with rich cellular infiltration. In some glomeruli there was an abundance of round-cells of the type described by Baehr. Some arteriolar showed thrombi and fibrinous excretions.

The pathologist (S. Fajers) considered the microscopical findings to coincide with those described for Libman-Sack's disease.

Discussion.

A pathological portrait is given of a middle-aged woman who has gone through various illnesses, inter alia, tuberculosis, but who felt fit and able to work the last five years until she fell ill in December, 1946, with insidious pains in the joints and muscles. For about two years she had, however, noticed acrocyanosis and a small eruption on one of her fingers. After the disappearance of fever and pains in the joints there occurred an accentuation of cutaneous changes. Examinations carried out four months after onset of illness produced evidence of certain symptoms of synovitis, hyperglobulinemia and anemia. The course of the disease was rapid and after about 4 months subsequent to the initial examination, 8 months after onset of the disease, resulted in death due to a clinical picture of a severe, general infection. The last month symptoms of polyserositis attended by high febrility occurred with concomitant further increase of the exanthema and the appearance of nephritic symptoms. Also petechias

were observable during some days as well as perivascular exudative nests in the retina. Autopsy verified the diagnosed polyserositis and revealed also a fat liver and nephritis. The histological examination showed changes in various portions of the peripheral circulatory system, the changes being mainly localized to the pre-capillaries, and of inflammatory type. Moreover characteristic changes in the lymphatic glands.

After a perusal of modern literature dealing with the conditions in the above disease known under various names, there are at least a few phenomena that can be established as characteristic of the clinical picture:

1. Its etiology is unknown (blood cultures neg.).

2. That the cutaneous manifestations *often* but *not always* existent, may vary in nature but nevertheless coincide with what is termed lupus erythematodes disseminatus in dermatological literature. That similar cutaneous changes may have the same etiology, for which reason lupus eryth. diss. would seem a somewhat incorrect or at least unsuitable term for this syndrome, it seeming more proper to view the cutaneous symptom as a partial phenomenon.

3. That articular manifestations, as is also the case with serositis, in one or more forms (polyserositis) belongs to the clinical picture.

4. That the onset is insidious and that fever is non-characteristic.

5. That various other symptoms often or at least not seldom attend the disease (anemia, nephritis, lymphadenitis, splenitis).

An inspection of biopsy results and a perusal of the descriptions of autopsy findings divulge the existence of a number of positive phenomena indicating the nature of the disease and elucidating its course. A number of authors do admittedly describe a special type of endocarditis, but this is rather the exception than the rule. Of great interest is the fact that *extensive histological changes in the peripheral vessels* were observable in a great number of cases. In 1935 Baehr et al. describes the condition as »A diffuse disease of the peripheral circulation». Non-specific pleuritis, pericarditis and peritonitis are often mentioned.

To the above and on the basis of his own case, and in the light of what is to be read in the literature, the author would add the following contemplations. Polyserositis-synovitis are the clinical manifestations that assume a predominant part of the picture.

Vasculitis, localized essentially to the pre-capillaries, but occurring also in the finer and coarser vessels is established, and it would seem that this phenomenon figures largely in the arisal of the motley picture (cutaneous symptoms, nephritis, anemia, retinal changes). Disturbances of the albuminal fraction of the blood have occasionally been mentioned earlier in the literature, but hyperglobulinemia and the nature of same have never been discussed. Is there not possibly some connection between these changes, and are not also changes in the blood a manifestation for an injury of the reticuloendothelium or are they not a reactive increase in the production of anti-body-globulin in response to an infection? The condition of the disease with the multiple localizations of the changes ought to fit very well into the picture of reticuloendothelial injury (vessels, liver, lymphatic glands, spleen and serous membranes). The course reminds one undoubtedly most of an infectious disease. The cause of the disease has not yet been established. In the author's case penicillin was absolutely ineffective. Finally it should be borne in mind that in cases of hyperglobulinemia of obscure genesis the Libman-Sack's syndrome ought not to be forgotten, especially in the presence of transient symptoms of the joints and short-lived cutaneous changes (with acrocyanosis) or such phenomena as retinal changes of doubtful genesis.

Summary.

The author describes and discusses a case of the Libman-Sack's syndrome in a woman of 45 years.

The course was rather acute. There were no signs of endocarditis.

The examination of the blood showed a considerable hyperglobulinemia and a pathological globulin of the γ -type. The ophthalmological examination showed signs of acute vasculitis in the retinal vessels. The treatment with penicillin was unsatisfactory. The diagnosis was verified at necropsy.

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The Reaction of the Liver to Small Doses of Vitamin K as a Liver Function Test.¹

(A Diagnostic Aid in the Differential Diagnosis Between Obstructive and Parenchymatous Jaundice.)

By

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(Submitted for publication December 16, 1947.)

One of the functions of the liver is the formation of prothrombin under co-operation of vitamin K.

In 1942 the authors suggested a method by which the prothrombin-forming capacity of the liver after administration of vitamin K may be used as a liver function test. Since the publication of that paper new numerical statements regarding the efficiency of the principle in question do not seem to have appeared, apart from a paper by Stein (1944).

In the present paper the value of the method for the differential diagnosis between obstructive and parenchymatous jaundice has been tested on a larger material than in the former and compared with the value of other liver function tests.

The Basis of the Method.

A condition for the performance of the test is a reduced prothrombin concentration in the blood. In total obstruction of the hepatic ducts such a reduction arises owing to defective absorption of fat soluble vitamin K (K avitaminosis stricte), whereas the reduction in cases of impairment of the liver parenchyma may

¹ The Study was supported by grants from "King Christian X's Foundation"

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also be caused by insufficient exploitation in the liver of the absorbed vitamin K; in hepatic cirrhosis it may also be that the absorbed vitamin K is not conducted through the porta-system (Kirk 1936).

By peroral administration of water-soluble substances with vitamin K effect (methylnaphtohydroquinones) a K avitaminosis in the strict sense will be cured independent of the presence of bile in the intestine, and the prothrombin percentage increase, if the prothrombin-forming capacity of the liver is unimpaired. In cases with impairment of the liver parenchyma the prothrombin percentage will not increase after administration of vitamin K or it will only increase very slightly owing to the liver's failing capacity to form prothrombin.

By administering suboptimal doses of water soluble vitamin K, which cause the prothrombin percentage to rise only to subnormal values, it is possible to register the maximum effect of the K-dosis administered (Begtrup & From Hansen 1942). In the present work, as in the previous, 2 mg Kvitasol¹ (methylnaphtohydroquinondisuccinate) have been used, a dose which has proved, in most cases, to be suboptimal.

Performance of the Test.

The concentration of plasmaprothrombin has been determined according to the method of Thordarson (1941), modified by Thordarson, Begtrup & From Hansen (1943).

Plasma-Prothrombin has been determined in 16 normal persons, the normal value being called 100 %. The mean and standard deviation have been calculated from the logarithmic distribution; the corresponding values in per cent are $s = 15$ %. Mean $\pm 2 \times$ standard deviation = 74—134 %. Our normal material thus has shown a greater standard deviation than that of Thordarson who found 85 % as the lower limit for normal values, wherefore in this work we have considered only values < 75 % as subnormal.

The prothrombin percentage of each examined blood-sample has been calculated by comparison with a blood-sample taken on the same day from one or the other of the two authors, whose prothrombin concentrations have been repeatedly compared without variations of importance in their mutual relations being

¹ The preparation was kindly placed at our disposal by "Lovens Kemiske Fabrik" Copenhagen.

found. This is in accordance with Thordarson's demonstration of the fact that in the individual normal person the concentration is constant over longer periods.

For determination of the «vitamin K sensitivity» («KS») a blood-sample is taken, then 2 mg Kvitasol are administered by mouth, and 24 hours later another blood-sample is taken. If in the patient examined 2 mg Kvitasol appears to be a suboptimal dose (*i. e.* the prothrombin content of the second blood sample is $< 75\%$), the possible rise in prothrombin represents the maximum response of the liver to this dose. In such cases the rise is denominated $KS = d$ (d : the difference between the prothrombin percentage in the second and first blood sample).

If on the other hand, it appears that the prothrombin content of the second blood sample is $\geq 75\%$, *i. e.* the prothrombin percentage has risen to values within the normal limits, 2 mg Kvitasol is not a definitely suboptimal dose, and the difference between the second and first prothrombin percent only represents the minimum response, and the rise determined is therefore denominated $KS \geq d$ (difference).

Authors' own Data.

The material comprises 152 patients admitted to different Copenhagen hospitals with verified diseases of the bile ducts or the liver parenchyma. The patients have been grouped as shown in Table I.

The diagnosis obstructive jaundice was ascertained by 1) Laparotomy or/and 2) post mortem examination. However, an odd case is included, in which the patient had been observed throughout a year and had had numerous typical attacks of gallstone with jaundice, but refused operation (1'. prothrombin percentage: 57 %, 2'. prothrombin percentage 113 %; $KS \geq 56$).

The diagnosis in the group «obstruction from other causes» have been: cancer of the ductus choledochus (one case), stricture of the ductus choledochus (one) and inflammatory edema of the pancreatic head and round the ductus choledochus (three), all verified by operation.

The diagnosis parenchymatous jaundice has been verified by: 1) Laparotomy or/and 2) post-mortem examination and 3) (the majority): a typical clinical course, in the case of acute hepatitis leading to absence of symptoms within three months (one patient

with acute yellow atrophy of the liver died). A few of the patients of the group: chronic hepatitis & cirrhosis of the liver were not jaundiced at the time when the KS was determined.

The data include only patients, in whom the prothrombin concentration of the first sample was $< 75\%$ and for all the patients only the first measured KS has been taken into account.

In table I the values determined for KS are divided into two groups: those, in which the second prothrombin percentage is $< 75\%$, KS in these cases being equal to the difference between second and first prothrombin percentage (upper row); and those, in which the second prothrombin percentage has appeared larger or equal to 75% , the difference ascertained in these cases being entered in the columns: $KS \geq$ (lower row).

Results.

From table I it appears, that KS was ≥ 30 in a great number of the cases with obstructive jaundice and < 30 in the great majority of the cases with parenchymatous jaundice; whereas $KS \geq 30$ and < 30 in patients with cancer of the liver, with or without occlusion of the bile ducts, were nearly equally represented.

In a fair many cases with cancer of the liver it appears that KS was < 30 . This must mean, that the prothrombin-forming activity of the liver in these cases has been reduced. Hence KS will have a low value irrespective of whether or not there also exists an obstruction of the bile ducts accompanied by K-avitaminosis, as is the case in the group: cancer of the caput pancreatis with metastases in the liver.

If KS is put $= 30$ as a diagnostic limit between the two forms of jaundice, obstructive and parenchymatous, the diagnosis obstruction of the bile ducts is confirmed in 77% of the cases and contradicted in 23% . (Table II columns a, b.) The diagnosis parenchymatous jaundice is confirmed in 86% and contradicted in 14% of the cases. The cases in which the KS is found to be \geq a value below 30 are left out of account in this calculation, as in such cases it is not known whether the real value of KS is above or below 30. The results obtained in such cases are considered »equivocal».

If $KS = 40$ is chosen as diagnostic limit the two forms of jaundice are confirmed respectively in 62% and 96% and contradicted in 38% and 4% of the cases, see table II, columns c, d.

Table II.

Numerical and Percentage Distribution of Unquestionable Values of KS.

	Number of patients (see table I)	Distribution of unequivocal results.							
		numerical (see table I)				percentage			
		< 30	≥ 30	< 40	≥ 40	< 30	≥ 30	< 40	≥ 40
Obstructive jaundice	36	7	23	10	16	23	77	38	62
Parenchymat. jaundice	99	75	12	79	3	86	14	96	4
Acute hepatitis	79	58	9	62	2	87	13	97	3
Chron. hepat. & hep. Cirrh.	20	17	3	17	1	85	15	94	6

a b c d

In patients with obstructive jaundice KS varies from 0 to ≥ 50 . No correlation has been found, as may be seen from table III, between the duration of the jaundice before the determination of KS and the value of KS; neither are low values of KS chiefly found in the cases, in which biliary cirrhosis has been ascertained by liver biopsy, operation or post-mortem examination. Probably poor response therefore may be due to a complicating, rather slight impairment of the parenchymal tissue arising from the bile stasis, or it may in part be due to a great individual variability in the resorption of the vitamin K, or in the response in cases with unimpaired hepatic function.

Table III.

Duration of Jaundice in Months Prior to the First KS Determination.

KS	< 1 mth.	≥ 1 mth.
< 30	4	3
≥ 30	11	12

Distribution of patients with occlusion of bile ducts according to KS and according to duration of jaundice prior to KS determination.

Results Obtained by other Authors.

Lord & Andrus (1940 and 1941), Allen & Julian (1942) and Stein (1944) all found better accordance between the K-tolerance test and the verified diagnoses than the present authors, as the first mentioned authors found the diagnosis confirmed in from 94 to 99 per cent of the cases obstructive as well as parenchymatous jaundice.

The said authors all use a larger dose of vitamin K than we do (Lord & Andrus, it is true, use the same dose, 2 mg, but their vitamin-K preparation (2 metyl, 1.4 naftoquinone), administered intramuscularly, probably has a stronger effect than ours). In the group of impairment of the parenchyma, we have found the same diagnostic accordance as these authors, but in the group obstructive jaundice the diagnostic accordance was less pronounced. It seems possible, even probable, that several of our cases with obstructive jaundice would have reacted more strongly to a larger dose of vitamin K without this dose having lead to a corresponding rise in patients with parenchymatous jaundice. This problem is being investigated by one of the present authors (H. B.). A defective resorption of the K-Vitamin given per os may probably also have influenced the result.

Comparative Investigations Regarding KS and Some other Liver Functions Test.

On some of the patients included here the respective hospital departments have performed various liver function tests such as Takata-Ara, urobilin determination in urine and galactose tolerance tests. Of each of these has been selected the test, which as regards time, is nearest to the KS first determined, including, however, only the patients, in whom the difference in time between the first KS and the selected test has been < 15 days. The frequency with which the above defined group diagnoses were confirmed by the various tests, has been calculated in Table IV.

For comparison have been entered in Table IV also calculations made on basis of the data published by Buch (1942); which comprise determinations of serum phosphatase and citric acid.

Table IV.

Practical Applicability in per cent	Liver function test	R e s u l t	Obstruc- tive jaundice	Parench. jaundice	Hepatit. ac.	Hepatit. chron. & Cirrh. hep.	Obstruc- tive jaund.	Parench. jaund.	Hep. ac.	Hep. chr. & Cirrh. hep.
56	KS(30)	$\geq 30 / < 30$	23/7	12/75	9/58	3/17	77	86	87	85
52	KS(40)	$\geq 40 / < 40$	16/10	3/79	2/62	1/17	62	96	97	94
100	Takata-Arn-test	- / +, + +, + + +	11/6	18/27	15/17	3/10	65	60	53	77
100	— » —	— + / + +, + + +	14/3	33/12	27/5	6/7	82	27	16	54
100	— » —	— +, + + / + + +	17/0	39/6	30/2	9/4	100	13	6	31
63	— » —	— / + + +	11/0	18/6	15/2	3/4	100	25	12	57
100	Urobilin in urine	—, + undil./ + indil. 1 : 4	19/5	45/21	39/18	6/3	79	32	32	33
100	Galactose toler- ance test	$< 3 \text{ g} / \geq 3 \text{ g}$	6/5	10/14	9/9	1/5	55	58	50	80
72	Serum phospho- tase & citric acid determi- nations	$\begin{array}{l} \text{Fo} > 10 \\ \text{Ci} < 27 \\ \text{Fo} < 15 \\ \text{Ci} < 30 \\ \text{Fo} > 20 \end{array} / \begin{array}{l} \text{Fo} < 10 \\ \text{Ci} > 27 \\ \text{Fo} < 20 \\ \text{Ci} > 30 \end{array}$	45/8	15/21	8/15	7/2	85	58	65	22
Buch's material										
a	b	c	d	e	f	g	h	i	k	l

Authors' material

Buch and the present authors have treated their data differently. In order to render the two materials comparable, Buch's data have therefore in the present work been dealt with on the same lines as given p. 33 and 35, *i. e.* only the first made tests in each patient of Buch's material have been included, and, furthermore, the frequency with which the diagnosis has been confirmed, is calculated solely on basis of unequivocal results of the tests. Buch's diagnoses have been made comparable with ours by dividing his cases into the groups obstructive and parenchymatous jaundice according to the criteria, which appear from p. 33 and Table I. By this means the frequencies with which all the function tests stated in the Table IV confirm the diagnosis obstructive jaundice, acute hepatitis and chronic hepatitis, respectively, have become mutually comparable.

When evaluating a liver function test, the applicability of the test ought to be considered separately from its reliability, *i. e.* the frequency with which it confirms the verified diagnosis.

The applicability of the test depends on how often it gives an (unequivocal) result, *i. e.* how often it confirms or contradicts a diagnosis verified by other means. If an appreciable number of the results cannot be said to give diagnostic information (equivocal result), or if the test cannot be used on an appreciable number of patients, its applicability decreases without its reliability being affected. Therefore, in order to evaluate the reliability, it seems most correct to reject the »equivocal» results and only calculate the frequency with which an »unequivocal» result either confirms or contradicts the verified diagnosis. (Vide Table II.)

Table IV. (Legend.)

Distribution of some liver function tests according to different criteria.

Column a: The frequency in percent with which the test in question can be used and/or gives decisive results («Practical applicability» of the test).

Column c: Numerator: The criterion chosen for the diagnosis of obstructive jaundice.

Denominator: The criterion chosen for the diagnosis of parenchymatous jaundice.

Column defg: The numerical distribution of the patients according to the stated diagnostic groups, using the criterion of column c.

Column hkl: The frequency (in per cent) into which the result of the liver function test in question confirms the stated diagnosis (diagnostic certainty). In the group obstructive jaundice (column h) the diagnosis is confirmed in the number of cases given in the numerator of column d. In the group parenchymatous jaundice (columns i, k, l) the diagnosis is confirmed in the number given in the denominator of columns e, c, f, g.

The practical applicability of KS (Table IV, column a.) has been calculated as follows:

$\frac{207}{326} \cdot \frac{134}{152} \cdot 100 = 56\%$ (limit 30), and $\frac{207}{326} \cdot \frac{124}{152} \cdot 100 = 52\%$ (limit 40), respectively.

The calculation is based on the following: 207 out of 326 (*i. e.* 63.5 %) patients with jaundice had a prothrombin percentage < 75 . 134 of the 152 KS-values determined were unequivocal, when 30 was reckoned as the diagnostic limit, whilst 124 values were unequivocal if 40 was chosen as diagnostic limit, *i. e.* 88 % and 82 %, respectively.

The Occurrence of Hypoprothrombinemia.

The frequency of subnormal prothrombin values, however, varies much according to diagnostic groups: In the 3 groups: obstructive, parenchymatous jaundice, and cancer of the liver, hypoprothrombinemia occurs in 49 %, 68 % and 88 %, respectively. The first group (obstruction) includes cancer of the head of pancreas without metastases to the liver, in which the frequency was 88 %, and cholelithiasis with a frequency of only 26 %. In group 2 (parenchymatous jaundice) hypoprothrombinemia are equally frequent in acute and chronic cases. In the said three groups, unequivocal results were found as follows (the first figure applies when 30 was chosen as limit, the figure in brackets when 40 was chosen): 83 % (72 %); 88 % (83 %); and 100 % (94 %).

If the two groups obstructive and parenchymatous jaundice are taken together, hypoprothrombinemia occurred in 177 out of 292 patients, *i. e.* 61 %, and unequivocal results of KS in 87 % (80 %, limit 40).

The reliability of a liver function test will vary in different diagnostic groups, *cf. e. g.* Table IV. If the different liver function tests are to be numerically evaluated as regards their value as differential diagnostics between obstructive and parenchymatous jaundice it is therefore necessary that all the different tests have been performed on the same patients; if it is not so, the frequency with which acute hepatitis occurs as compared with chronic hepatitis will influence the affirmative value for the group: parenchymatous jaundice.

In the present comparison this demand has not been gratified, wherefore the values in col. i, Table IV must be compared with due reservation.

As in both hepatitis acuta and hepatitis chronica (col. k. and l, Table IV) application of the other liver function tests, however, leads to lower figures for the affirmative value than KS, parenchymatous jaundice will present the larger affirmative value with the KS test, regardless of the ratio between acute and chronic hepatitis.

Conclusions.

These comparative investigations presented here, show that the agreement with the verified diagnosis is better with the K-tolerance test, than with any of the other liver function tests examined, even though its application is limited primarily to cases with a reduction in blood prothrombin.

Summary.

The prothrombin-forming activity of the liver following administration of vitamin K is used as a liver function test (called vitamin-K sensitivity, »KS«, by which is understood the increase in prothrombin percentage 24 hours after administration of 2 mg Kvitazol).

152 cases of jaundice with verified diagnoses have been examined. Of these 36 were obstructive and 99 parenchymatous jaundice, while 17 had cancer of the liver. Only the first KS determined in each patient is taken into account.

$KS \geq 30$ confirms the diagnosis obstructive jaundice in 77 % of the cases, while $KS < 30$ confirms the diagnosis parenchymatous jaundice in 86 %. If 40 is chosen as limit the two figures change to 62 % and 96 %. The almost corresponding percentages for the Takata-Ara test in nearly the same material are 65 % (—) and 60 % (+, ++, +++), for urobilin excretion 79 % and 32 %; for galactose tolerance tests 55 % and 58 %, and finally Buch's data, having been dealt with in accordance with the rules used in this paper, showed 85 % and 58 % for combined serum phosphatase and citric acid determinations.

For chronic impairment of the parenchymal tissue (chronic hepatitis and cirrhosis of the liver) the percentages are found to be: KS: 85 %, resp. 94 %, Takata-Ara: 77 %, urobilin: 33 %, galactose: 80 % and combined serum phosphatase & citric acid: 22 %.

The greater diagnostic certainty of KS, as compared with other liver function tests examined is demonstrated.

The authors wish to express their thanks to Professor, Dr. med. E. Meulengracht for valuable discussions on the subject of this paper.

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Spontaneous Hypoglycemia.

Considerations on Indications and Result of Operation.

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The diagnosis of organic hyperinsulinism in patients with spontaneous hypoglycemia is based upon:

1. a fasting blood sugar level below 50 mg% by repeated observations after normal diet;
2. attacks of severe neurological or psychiatric disturbances and a low blood-sugar level;
3. prompt relief from the attacks by administration of sugar;
4. exclusion of hepatic, adrenal, hypophyseal and neurological lesions.

If these criteria are found in a patient, operation is considered to be advisable. In 75 % of the operated cases an adenoma of the pancreas is found. If no adenoma is found, a non palpable adenoma or a general hyperplasia of the Islets of Langerhand may be the cause of the attacks.

Therefore, if no tumor is found, partial or subtotal pancreatectomy is advised by several authors.

David¹ reports 18 cases of partial resection of the pancreas, wherein 18—24 gram pancreatic tissue was removed: 15 times the tissue was normal, and three cases showed hyperplastic islets.

¹ David. Indications and results of pancreatectomy for hypoglycemia. Surgery, St Louis 8, 212, 1940 (Ref. J. A. M. A. 115, 1313, 1940).

Three patients were cured, three showed marked improvement, and eight remained unimproved. In another series of 17 patients, 35—60 grams of pancreatic tissue was removed which showed no tumor: in 14 cases the tissue was normal, in two cases hyperplastic, and in one case there was pancreatitis. One patient succumbed to the operation, 11 were afterwards free of symptoms, and four showed no improvement.

David concludes that if, in cases with the clinical diagnosis of organic hyperinsulinismus no adenoma is found, a subtotal pancreatectomy is indicated. Bernstein¹ is of the same opinion. Though this conclusion may in most cases be correct, it is also true that clinical recovery may be obtained in another way. Two cases are presented; in the first, a small piece of pancreatic tissue was removed at operation, which on histological examination appeared to be normal. The patient had an uneventful recovery. In the second case, the patient developed very severe attacks, a high degree of clinical improvement was obtained without any operation.

1. *Severe hypoglycemic attacks; explorative operation; removal of a small piece ($\pm 3 \times 4$ mm) of the pancreas (normal tissue); recovery.*

Patient A., a 52 years old male, consulted us (viz. v. B.) three and a half years ago. At that time he had complained, for about three years, of impaired vision always before breakfast. He could not read, nor could he, in the street, recognize his acquaintances; however, following breakfast vision always returned to normal.

He had never been ill except for symptoms of duodenal ulcer in 1932 (hunger-pain and melaena).

Six weeks before admission to the hospital he had attacks of profuse perspiration about 4 to 5 o'clock in the morning. He also perceived that this symptom disappeared immediately after eating some bread. Soon the attacks began to appear during the day, and he was forced to take food more frequently which in turn resulted in a gain of 5 kg in body weight in six weeks.

On the morning of July 22nd, 1944, the patient's wife observed that he made some peculiar sounds. He imitated the sounds he heard, stared vacantly, swore (what he never otherwise did), and made stereotype movements with hands and feet. In the beginning he refused to take food but finally he was made to swallow some porridge, and immediately recovered having no memory of the attack.

The next morning a similar seizure occurred, and admission to the hospital followed. Here also he had several analogous attacks, which

¹ Bernstein. Journ. Mount Sinai Hosp. 12, 66, 1945.

often resulted in coma. During these attacks the level of the blood sugar was low but administration of carbohydrates per os or by intravenous injection of glucose solution, were promptly followed by recovery.

The fasting level of the blood sugar was:

- 25 July 55 mg% (8 hours after the last meal): profuse perspiration.
- 26 July 44 mg% (12 hours after the last meal): patient disorientated.
- 27 July 78 mg% (3 hours after the last meal): no symptoms.
- 28 July 55 mg% (7 hours after the last meal): profuse perspiration.
- 30 July 37 mg% (12 hours after the last meal): stupor.
- 31 July 59 mg% (6 hours after the last meal): severe perspiration.

After 50 gram glucose per os the blood sugar was:

After 1 hour, 113 mg%.

After 2 hours, 70 mg%.

After 3 hours, 59 mg%.

After 4 hours, 55 mg%.

On July 27th a blood sugar curve was taken three hours after the last meal.

First determination 78 mg%.

20 minutes after 50 g glucose 151 mg%.

40 minutes after 50 g glucose 137 mg%.

60 minutes after 50 g glucose 119 mg%.

180 minutes after 50 g glucose 42 mg%. (no symptoms).

The urine never contained sugar.

On July 29th the patient was given, on three occasions during the night, a slice of bread because of perspiration; the last attack was at 4 o'clock in the morning. At 7.30 a. m. he felt well, and attempted to rise but fell immediately on the floor, exhibiting a right side paresis of the arm and leg; the Babinskis sign was positive. There was no perspiration, no disorientation, and no retrograde amnesia. One hour after breakfast and an intravenous glucose injection the strength in the right arm and leg returned; after four hours the right abdominal reflex was still diminished but in the afternoon the blood sugar was 66 mg%.

General examination of this normal, strong man, with a well-balanced personality, showed no abnormalities. The fundus oculi was normal on both sides, visual acuity was 5/5 and vision for motion and colour was quite normal. Liver function: no urobilinuria, the bilirubin content of the plasma was 1 U (Hijmans van den Bergh); reaction of Takata-Ara showed flocculation in one tube and the galactose test showed no reduction in the urine; Basal metabolism was + 20. Blood examination showed calcium 9 mg%; the leucocytes were 7,700 with eosinophils 4 %, stab 4 %, segment neutrophils 73 %, lymphocytes 7 % and monocytes 2 %.

The feces showed no indigested particles.

At home the patient was given about 400 g of carbohydrates daily and while in the hospital, a diet of 240 gram carbohydrates per day was followed by a higher frequency of the attacks.

Obviously the patient was exhibiting attacks of spontaneous hypoglycemia, accompanied by serious disturbances of neurological and psy-

chical character. The fasting blood-sugar level was very low, the attacks were promptly relieved by administration of carbohydrates and the patient developed fewer attacks following a high carbohydrate diet.

All the signs were present which according to Wilder, Conn, and others prove the organic character of the spontaneous hypoglycemia.

There were no arguments in favour of a disturbance in the liver function, or of an insufficiency of the hypophysis or the adrenals, nor of an abnormality in the hypothalamic region. The only explanation seemed to be an organic hyperinsulinism.

In view of the rapid development of the sickness, operation followed.

At the operation (Dr. Karthaus) the gallbladder, liver and pancreas appeared to be quite normal. The cauda and the caput of the pancreas were prepared free as far as possible, a small induration was removed. After a search of two hours and a half the operation was terminated.

In the piece removed Prof. Gaillard (Leiden) found only normal pancreatic tissue.

The first three days after operation severe shock prevailed, which necessitated several blood-transfusions. A total four and a half of liters was transfused. The hyperglycemia during the first days was combated with insulin.

Since the operation three years and a half have passed and during this time the patient has been free of attacks. Very shortly post operation he was eating only three times a day and in the morning before breakfast no symptoms are present. The fasting level of the blood-sugar on repeated examinations was 90—95 mg%. Also after a diet poor in carbohydrates for some days a normal blood-sugar was noted.

It is obvious that after the operation this patient recovered from the illness, which we must consider as an organic hyperinsulinism. Similar course has been described more than once.¹

With recovery following an operation with manipulations of the pancreas, the pancreatogenic cause of the hypoglycemia-attacks would appear to be evident.

More frequent than recovery after a negative exploration are the cases where no improvement was observed. Several times an adenoma was found at a second operation.

¹ Holman a. o. Arch. Surg. 47, 165, 1943.

Looking for an explanation there would seem to be two possibilities:

1) The removal of the small piece of pancreatic tissue was sufficient to reduce the hyperactivity of the pancreas;

2) perhaps the manipulations during the operation led to necrosis of a not detected homo- or heterotopic adenoma.

Not only local necrosis, but also an acute general pancreatic necrosis as a result of the operation should be taken into account.

Though we have no sufficient reasons to justify this explanation, some postoperative symptoms are further arguments for this supposition, namely: the shock lasted very long, hyperglycemia existed after operation, the patient had severe pain in the left upper abdomen and the breast, which lasted a long time and was accompanied by a slightly higher temperature during three weeks; in the left lung there was a slight dullness with diminished respiration, without signs of thrombosis or emboli (the diastase content of the urine was not determined.)

This case shows that some thought should be given to the indications for extensive operation following a negative exploration.

The above-mentioned results of subtotal pancreatectomy were but moderately favourable. The suggestion of Waugh¹ of a total pancreatectomy would seem not to be advisable, though Waugh performed this operation twice with success. He advises to resect first cauda and caput (where 80 % of the adenomas are found), and in the absence of a palpable tumor, to remove the rest of the pancreas immediately.

However three considerations seem to oppose this suggestion. Firstly a non palpable, microscopic adenoma may be present in the cauda or caput.

Secondly, the definite disturbance of the intrinsic and extrinsic secretion of the pancreas should be prevented, unless absolutely necessary. Without a careful examination of the removed tissue, and a control of the postoperative course this necessity cannot be accepted.

Thirdly, at least six cases have been published where organic hyperinsulinism was due to an adenoma or carcinoma in dystopic pancreatic tissue. This tissue lies as a rule in the stomach, the duodenum or the jejunum, and sometimes elsewhere.²

¹ Waugh. Proceedings Staff Meetings Mayo Clinic, 21, 25, 1946.

² Zaslow and Connors. Proc. Mayo Clinic 21, 241, 1946.

Finally, it is certain that cases of organic hyperinsulinism occur, where neither at the operation nor at the autopsy a satisfactory explanation can be found for the hypoglycemic attacks.¹ Our second case shows that without any operation considerable improvement may sometimes be obtained in cases in which signs and symptoms seem to prove the organic character of the hypoglycemic attacks.

2. Severe and temporary strong progressive hypoglycemic attacks, disappearing under conservative therapy.

Mrs. B., born 11-6-1904, had symptoms which appeared as early as 1931. In December 1931, two months after her *first confinement* at 11.30 in the morning following a walk she developed a prickling sensation in the face and hands, her sense of taste was lost and her tongue was somewhat thickened. During the seven months she nursed her child these symptoms appeared at intervals. After the *second confinement* (February 1933) these symptoms recurred after rising in the mornings during the seven months she nursed her child. In December 1933 the patient was upon for operated extra-uterine pregnancy and post-operatively in January 1934, felt the same sensation of prickling in the face and tongue.

She became pregnant again, and in a few months the symptoms returned in a severer form resulting in a numbness of the face, diminished sense of taste, and a thick tongue; these sensations recurred several times a day persisting for about half an hour with each attack.

On December 29th, 1934, the *third child* was born, and two days later the symptoms in the hands, face and tongue occurred first time after this confinement. She was very tired. The symptoms returned in the days following, and were more pronounced: she became irrational, her tongue was thick and her face was flushed. She had difficulty in seeing and diplopia was present. Taste was totally absent for an hour.

After January 12th cramps appeared in both arms and legs. On January 16th, after nursing her child, she lapsed into a coma dropping her child and became incontinent of urine.

On January 18th she was admitted to a hospital, where she ceased nursing the child. Next morning at 8.20 the same sensation returned, during fasting for a spinal puncture, she did not feel well, asked for breakfast, later she again developed diplopia.

Soon she had a convulsive seizure of the right side, had tingling sensations in the neck and face and became restless and anxious. Her pulse was poor and irregular. In the cerebro-spinal fluid no abnormalities were noted (except some blood from the puncture.)

The temperature was normal.

The catheter urine contained albumin and a few leucocytes.

Blood: Urea 54 mg%, calcium 10.3 mg%. Hb 65 %, leucocytes 6,900, with normal differentiation.

¹ Kepler. J. A. M. A. 115, 1675, 1940.

Fasting blood sugar: 55 mg %; after breakfast 88 mg%.

Similar attacks as described returned, and were taken for epileptic equivalents; as a result luminal was administered.

On January 22nd, a severe attack occurred in the morning with a feeling of suffocation, anxiousness, motoric restlessness and a weak pulse.

As an epileptic attack was supposed, no sugar was given; and the attack persisted. At 11.30 the neurologist diagnosed a spastic paralysis of both arms and legs with a positive Babinski's sign on both sides. The respirations were superficial, and sometimes ceased momentarily. At 2 p. m. after sleeping 15 minutes, she was suddenly much better, had hunger, took some food, and could recount the beginning of the attack.

From this time her condition improved; she was treated with luminal and left the hospital on March 14th.

At home she was treated with luminal at intervals and had only slight symptoms.

On May 27th, 1937 a severe attack occurred; and luminal therapy was again instituted. In 1942, after a case of erysipelas, the attacks became more frequent, and she consulted us (viz. L) on March 21st. The neurologist and the physician still regarded her condition as epilepsy. But the patient knew that the attack appeared before breakfast and disappeared after she had eaten, she was accustomed to have breakfast ready at the bedside, and to take it immediately upon waking. Some days earlier she awoke, took no breakfast but fell asleep again, and her husband could not awake her. She was stiff, kept her mouth closed, and it was five o'clock in the afternoon before she could talk. This occurred a day after a gastro-enteritis, and was considered to be a status epilepticus.

As a rule she awakes in good condition, but she knows that it is dangerous for her to make any movements (c. g. to dress her hair) before eating.

The menstrual periods occurred every three weeks, but became less severe following her last confinement.

Examination showed no major abnormalities. The hairgrowth in the axillae and the regio pubica was normal. Blood-pressure was 105/70 in the recumbent and standing positions.

On vaginal examination a normal uterus in the anteflexed position was palpated.

The sella turcica was normal on roentgen examination.

Blood: Sedimentation rate 20 mm, Hb. 85 %, erythrocytes 4.1 million, leucocytes 5,600, with 1 stab, 41 segmented granulocytes, 41 lymphocytes, and 3 monocytes per hundred.

Cholesteroline: 190 mg%. Basal metabolic rate: — 8.1.

Feces: no abnormalities. Patient was admitted to a hospital for observation.

On April 8th a blood-sugar curve was planned, for which purpose she was obliged to fast. At 7 o'clock she was very restless, at 8 o'clock she was more quiet and was in a kind of stupor. At that moment the fasting blood sugar was 76 mg%.

A quarter of an hour after the ingestion of 50 gram glucose she was quite well: blood sugar 119, 143, 123, 115 mg% at intervals of half an hour (method after Terwen).

April 11th: Fasting blood sugar was 61 mg%, after washing herself it was 60 mg%. Five minutes later she became stuporous and restless, exhibiting jactio, grimaces and negativism; she refuses sugar and ingestion had to be enforced.

April 15th: blood sugar: fasting 109 mg%, after washing 60 mg% and 67 mg%. She was moderately well, but not entirely normal.

April 20th: Blood sugar estimation after adrenaline injection was planned. Fasting level was 88 mg% (7 hours 15), 7 h. 20: 1 mg adrenaline subcutaneously. 7 h. 35 blood sugar: 83 mg%; a severe attack developed; she became very restless, plucked at the blankets, tearing her clothes, sniffed, growled, flinging herself to right and left. At 7.50, a sugar solution was introduced by a tube through the nose.

8 h. 5: blood sugar: 86 mg%. Thus there was no reaction on adrenaline. April 22nd. Adrenaline-test in the afternoon.

3 h. 20 blood sugar 121 mg% (injection of 1 mg adrenaline)

3 h. 40 " " 122 "

3 h. 50 " " 125 "

4 h. 00 " " 119 "

Though she felt the action of the drug very well (palpitations etc.), there was no effect upon the level of the blood sugar. Although we thought that operation was justified in this obvious case of organic hyperinsulinism, on account of war-conditions laparotomy was not performed, but tentative conservative therapy was instituted.

She was instructed to wake herself with an alarm-clock at 5 a. m. and after taking some food to return to sleep. During daytime she should take frequent meals, which were rich in carbohydrates (a protein-rich diet had no favourable effect). With this regimen she improved. Post-pituitary extract and ephedrine were administered, but this therapy did not seem to be essential.

A few times, when she did not hear the alarm-clock, she became comatous by morning, but when instructions were followed exactly, she had no complaints.

In March 1947 she was admitted to a private clinic for observation. She had minor gastric complaints, but no ulcer was found. After the end of the war, with the better food conditions, she improved still more.

March 13th blood sugar fasting condition: 54 mg% (Folin and Wu).

$\frac{1}{2}$ hour after 50 g glucose: 101 mg%

1 " " " " 107 mg%.

$1\frac{1}{2}$ " " " " 113 mg%.

2 " " " " 105 mg %.

$2\frac{1}{2}$ " " " " 82 mg%.

3 " " " " 92 mg%.

$3\frac{1}{2}$ " " " " 68 mg%.

4 " " " " 55 mg%.

March 14th blood sugar in fasting condition: 42 mg%.

This patient has a long history (of 15 years) of attacks of spontaneous hypoglycemia, which for 10 years had been considered as epileptic attacks. These seizures occurred three times in the puerperium after her three confinements, and again during the war, when the food supply was insufficient.

At last examination the fasting blood sugar level was still 42 mg% (Folin and Wu). During the observation in 1942 the lowest value was 60 mg% but this was estimated after Terwen, and it is probable that this method does not yield wholly reliable results for the lower values. A value of 83 mg%, however, during a severe attack was very remarkable. Joslin¹ does not remember a hypoglycemic result with a blood sugar value above 80 mg%. Several authors, however, observed a very low blood-sugar without symptoms, and the development of an attack when the blood sugar was again rising.² There is no absolute parallelism between the clinical and the biochemical condition.

The outbreak of spontaneous hypoglycemia during lactation is well known, Stenström described³ two cases. Also in the case of Pompe⁴ the symptoms developed twice, 13 and nine days respectively after confinement; afterwards an adenoma of the pancreas was removed. Pompe *c. s.* concluded to a decreased sensitiveness towards an excess of insulin during pregnancy, lasting for a short time after confinement.

However, the period of latency in our case was much shorter (2 days). We consider the attacks of our patient originated rather from an organic than from functional disorder. The diminished menstrual blood-flow suggests the influence of the hypophysis, but menstruation appears every three weeks, and no other hypophysical symptoms were present. Therefore we think it more probable that this patient has an adenoma of the islets of Langerhans.

It is noteworthy that clinical recovery could be obtained without operation, though biochemically the condition remains unaltered. This shows that hesitancy to operate is advisable in some cases.

¹ Joslin. Treatment of diabetes.

² Kolff *Geneseskundige Gids* 1944, no 2, 3 en 4 (Jan.—Febr.).

³ Stenström. *Dtsch. Arch. Klin. Med.* 153, 181, 1926.

⁴ Pompe, Jansen and Dhont. Adenoma of the islets of Langerhans and pregnancy. *Acta Med. Scand.* 124, 334, 1946.

Summary.

Two cases of hypoglycemic hyperinsulinism are presented. The first recovered after a purely explorative operation, whereby only a very small piece of normal pancreatic issue was removed; the second recovered clinically, though not biochemically, without any operation under a conservative regimen.

The indications and the results of operation are discussed.

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From Ullevaal Hospital, IXth (Medical) Department, Oslo.
(Chief: H. J. Ustvedt, M. D.)

Erythema Exudativum Multiforme Viewed from an Internal Medical Standpoint.

By

HANS JACOB USTVEDT.

(Submitted for publication January 10, 1948.)

III.

Etiological Possibilities Apart from Tuberculosis.

In a previous article it has been shown that in this material comprising 202 cases of E. multiforme, one can reckon with the probability or possibility of tuberculous etiology in from 35 to 40 cases, *i. e.*, in about one-fifth of the total number. It cannot be denied that a small number of the other cases may also have etiological connection with tuberculous infection, for example, cases with vesicular tuberculin reaction and negative X-ray findings and in which gastric lavage has not been performed.

Etiologically, however, E. multiforme as a whole seems to differ clearly from E. nodosum. For the latter exanthema a tuberculous etiology is the general rule (at least 50—60 %), while for the former such etiology is found only in a minority of cases.

In the discussion respecting the etiology of E. multiforme the question of «*rheumatic infection*» has always been in the foreground. C. Boeck in 1877 noted in a familial epidemic of sore throat that one member of the family got rheumatismus acutus, while another member had erythema nodosum, and he regarded both E. nodosum and E. multiforme as being forms of «*skin rheumatism*». In more recent years it is especially the etiological importance of the streptococci that has been most discussed.

It has earlier been mentioned that *pains in the joints* formed a prominent feature in the prodromes in the group with combined

E. m. and E. n., while they were extremely seldom present in the prodromal stage of the mucous membrane affections. The same applied to dysphagia. If we further consider the frequency of joint pains in the later course of the disease, it will be seen that such pains occurred in about half of the cases of combined E. m. and E. n., but only in about 1—7th of the other cases. The dysphagia not infrequently seen in patients with mucous membrane affection can hardly have been due to angina, but probably to an affection of the mucous membrane in the pharynx, of the same nature as the stomatitis.

The group with combination of E. m. and E. n. thus presents as regards the occurrence of joint pains a somewhat similar picture to that seen in cases of pure E. nodosum (Rotnes, Scheel, Skiöld, Löfgren).

I have mentioned that some of the patients with E. m. were admitted to the hospital under the diagnosis *rheumatismus acutus*. In a large number of cases the diagnosis at the time of discharge was the same, in addition to E. multiforme. Opinions may, of course, differ as to the criteria on which the diagnosis *rheumatismus acutus* or *polyarthritis acuta* shall be based, and the matter is still more uncertain if we introduce the term *febris rheumatica*. By the name *rheumatismus acutus* I have here chosen to designate those diseases that are characterized by pains occurring successively in a number of joints, combined with distinct swelling of one or more joints and a considerable rise in the sedimentation rate. I have not demanded the presence of hemolytic streptococci in the throat or electrocardiographic changes, although such findings have been made in some of the cases. In most cases an attack of angina has preceded the onset of the illness. Several of the patients have had acute rheumatism one or more times previously and have stated that during the attack of E. multiforme the rheumatic affection had exactly the same course as before. Many of the patients have proved to be remarkably amenable to salicyl treatment. I think it may be permitted to say that, if E. multiforme had not been present at the same time in these cases, the diagnosis would undoubtedly have been *rheumatismus acutus*. I do not here mean to express any opinion as to whether these cases are of the same nature, etiologically and pathogenetically, as the »true» rheumatic fever.

In the entire material there was found at the same time acute rheumatism with typical picture in 19 cases, somewhat atypical

in 12, i. e., in altogether 31 cases, or about 15 per cent. Here, as in the case of the joint pains, there was a striking difference between the groups. In the mucous membrane group only 3 cases showed a typical and 3 an atypical picture of acute rheumatism, making together 7.5 per cent, while the group with unimixed E. m. and the group with combined E. m. and E. n. had respectively 18 per cent (9 typical, 3 atypical) and 20 per cent (6 typical and 4 atypical cases). The mucous membrane group, which can hardly be deemed to have any relation to tuberculosis, shows at the same time least clinical association with rheumatismus acutus.

Edström finds simultaneous occurrence of acute articular rheumatism and E. nodosum in 22.5 per cent of his cases, Josephsen in 33 cases out of 71. Löfgren finds swollen joints in 27 per cent, Sköld in only 6.7 per cent.

Accordingly we see that alike in pure E. n. and pure E. m., as well as in combined cases, both joint pains and swelling of one or more joints, with a clinical picture resembling acute rheumatism, seem to occur with considerable frequency. This characteristic clinical finding, however, does not tell us much about the etiology. A number of authors, including Rönnes and Schöel, have shown that the affections of the joints in E. nodosum occur with great frequency in cases due to tuberculous primary infection. Rönnes finds joint troubles in 42 per cent of his patients with E. nodosum, and he points out that most of them had previously never had rheumatic disease. Rönnes regards practically all his cases as being due to tuberculosis.

Acute polyarthritits of the same clinical type as the »true» polyarthritits rheumatica has been found to occur in case of tuberculous primary infection, for example, by Heimbeck, Öwren, Ustvedt. Thus it is clearly established that neither pronounced articular pains nor the clinical picture of polyarthritits acuta together with E. nodosum can be said to preclude the possibility of tuberculous etiology. As regards E. multiforme, the present material includes 4 cases in which the exanthema was accompanied by acute polyarthritits, while at the same time the presence of tuberculous primary infection was indicated by conversion of the tuberculin reaction and by detection of tubercle bacilli in the gastric lavage fluid.

It is further seen that no help is afforded by the electrocardiogram when it is a question of distinguishing a »true rheumatic polyarthritits» in cases of E. multiforme or E. nodosum from a

clinical picture resembling »acute rheumatism», due to tuberculosis. In *E. nodosum* Löfgren found signs of fresh myocardial changes in 5 patients out of 178, and tuberculous primary infection existed in three of these cases. Owren has reported a case of fresh tuberculosis with myocardial changes.

In the present material signs of *endocarditis* have not been noted in any of the cases during the course of the disease. In two cases, both of the rheumatismus acutus type, *pericarditis* was observed, and in five cases there were recorded electrocardiographic changes which might be taken to indicate a fresh *myocardial lesion* (in 4 cases lengthened PQ interval, in one case Wenckebach periods). Altogether 35 patients were subjected to electrocardiographic examination. In one case with verified tuberculous primary infection (Tbc. +) a PQ interval of 0.32 sec. was temporarily found.

Thus it holds good both for *E. m.* and *E. n.* that some of the clinical pictures suggestive of acute rheumatism are due to tuberculous primary infection. On the other hand, however, we find them at least equally often in the *tuberculin-negative cases*. After Löfgren's demonstration of the etiological importance of the hemolytic streptococci in some cases of *E. nodosum* and *E. multiforme* it might seem natural to suppose that the affections of the joints in these exanthemas were ascribable to infection by streptococci, either alone or together with tubercle bacilli. Meanwhile it appears that the explanation is not so simple. Both in Löfgren's *E. nodosum* material and in the present collection of *E. multiforme* patients we find cases with pronounced symptoms from the joints, where hemolytic streptococci cannot be detected in the fauces, where the antistreptolysin titer is normal and the skin reaction to emulsions of hemolytic streptococci is negative. Löfgren points to the interesting finding in his material that painful joints occur in 45 per cent of the cases with tuberculous etiology, in 65 per cent of the cases due to streptococcal infection and in 77 per cent of the cases with *uncertain etiology*. Painful joints are especially prominent in some cases with bilateral hilar adenitis (sometimes with erythema nodosum) and with negative or weak tuberculin reaction, where no signs of infection by streptococci can be detected.

It is reasonable to suppose that both tubercle bacilli and streptococci, as well as unknown factors of infectious or allergic nature, can give rise to the occurrence of joint phenomena in *E. multi-*

forme. As example of a case with signs of streptococcal infection the following may be mentioned:

Woman aged 39. Had erythema nodosum at the age of 7 and of 15 years. After a week's dysphagia and joint pains came bullous erythema multiforme. Continued wandering pains in the joints with swelling in several joints. Pirquet vesicular. Radiogram of lungs: calcified primary complex. Gastric lavage: Tbc. — Fauces: pure culture of hemolytic streptococci. A. S. T.: 400. Skin test with hemolytic streptococci: vesicular reaction.

As to what factors come into play where no signs of tuberculosis or of streptococcal infection can be found we know very little. The theory of a specific rheumatic virus cannot be said to have been abandoned. Can it in some of these cases be a question of a *genuine rheumatic infection*, accompanied by E. multiforme? Against such an assumption speaks the extremely rare occurrence of valvular disease.

Taking as starting-point a material comprising cases of polyarthritis acuta, we find that exanthemas occur with very great frequency. Among 226 polyarthritis patients treated in Ullevaal Hospital. Dept. IX, in the period 1925/35 I found 8 cases in which E. nodosum and 5 in which E. multiforme was present at the same time, as well as 12 patients with uncharacteristic exanthemas, whereof some were possibly cases of E. multiforme. Thus we find exanthemas in over 10 per cent of the whole material. It is possible, however, that the cases with E. nodosum or E. multiforme did not represent genuine rheumatic infections. Among 850 cases of polyarthritis Edström found E. nodosum at the same time in about 6 per cent. On re-examination of 48 patients with simultaneous polyarthritis and E. nodosum he found undoubted vitium cordis in 23 cases, which would seem to indicate that also the true rheumatic infection can give rise to E. nodosum. Meanwhile the different authors report conflicting findings in this respect and the question has not been made clear.

In this connection we come to the question of the etiological importance of *the streptococci* in E. multiforme. Löfgren has established that the streptococci, to some extent in collaboration with tubercle bacilli, are of etiological significance in many cases of E. nodosum and he made the same observation in two cases of E. multiforme. In the present material the rôle played by the streptococci has been investigated only in some few cases from recent years, and therefore the material, apart from some individual ob-

servations, cannot to any appreciable extent throw light upon the question of the significance of the streptococci.

In 22 cases we have direct or indirect evidence of a connection with streptococcal infection, seeing that the exanthema appeared in conjunction with angina phlegmonosa, streptococc-otitis or lymphadenitis, or else there was found elevated A. S. T. and pure culture of hemolytic streptococci in the throat, without signs of other etiology. The actual number of cases due to streptococcal infection would probably have been found to be considerably higher, if this matter had been systematically investigated.

I shall briefly go through the different groups with a view to the etiological possibilities that may come into question.

In 22 out of 31 cases with manifestations from *several* mucous membranes no indications whatever were found respecting the etiology. In one case streptococci were obtained in pure culture from the oral secretion (stomatitis) and in another case streptococci were found in pus from suppurative otitis, but these findings furnish no proofs at all respecting the etiological importance of the streptococci. In one case the disease arose in conjunction to a sublingual panaritium, together with furunculosis, and here it may well be imagined that staphylococci played a rôle also as regards the occurrence of E. multiforme.

In the earlier described case, with fatal issue, of a 6-year-old girl both parents had syphilis. In another case E. m. appeared simultaneously with a secondary syphilitic exanthema. No indubitable evidence of tuberculous etiology was found in any of the cases in this group.

In one patient, a child of $2\frac{1}{2}$ years, the disease set in after the child had been given 0.05 g of *luminal* during a week and a half. S. Bache has reported two cases of fatally resulting luminal intoxication in children, presenting the picture of E. multiforme with manifestations from mucous membranes. In one case the exanthema appeared one hour after the patient had received 0.5 g of *sulphapyridine*, in another case there came exacerbation of the exanthema after treatment with *sulphathiazol*. One case arose during *salvarsan* treatment for neurosyphilis.

The etiological correlation must in these cases be described as being in the highest degree uncertain, possibly with exception of the luminal intoxication. Thus it seems to be characteristic of the group of cases with multiple manifestations from mucous membranes that the evidence they offer as regards etiological factors

is extremely small. I lay special stress upon the fact that tuberculous primary infection was not noted in any of the cases and that none of them presented the picture of acute rheumatism.

Among the 34 patients in whom *isolated stomatitis* occurred together with the exanthema there were in three cases found indications of streptococcal affection: 1) angina phlegmonosa, 2) pronounced symptoms from the joints, growth of green streptococci in blood agar and from a dental abscess, 3) pure culture of hemolytic streptococci from the throat, A. S. T. 400, vesicular skin reaction. In one case the disease appeared in immediate conjunction with sulphathiazol treatment of angina.

In three cases there was noted a doubtful connection with tuberculous primary infection, in one case the syndrome: bilateral hilar adenitis, painful joints and negative tuberculin reaction (which Löfgren calls the B. H. L. syndrome), while one case presented the clinical picture of acute rheumatism. In the remaining 25 cases no etiological data whatever were found.

For the whole group of 65 patients with typical mucous membrane manifestations we thus find more or less reliable evidence of streptococcal or staphylococcal infection in 6 (7) cases, uncertain evidence of syphilitic genesis in 2 cases and possibility of medicamental influence in 4 cases, *while the vast majority of the cases offer no data whatsoever of etiological nature.*

It has already been mentioned that the small group of 15 patients with *isolated affection of the eyes* occupies a special position and has points of contact with the cases where E. m. occurs in combination with E. n. Here there were found indications of tuberculous primary infection in 4 cases, of streptococcal infection in 4 cases and of staphylococcal infection in one case, while two presented the picture of rheumatismus acutus, without signs of tuberculous or streptococcal infection. We are here entirely without etiological data only in 4 cases.

In the group of 67 patients with *pure E. multiforme without manifestations from mucous membranes* the etiological possibilities are distributed as follows:

Probable tuberculous genesis	6 cases
Possible » »	5 »
Streptococcal and staphylococcal infections	8 »
Effects of sulphonamide	3 »
Salvarsan treatment	2 »
Allergic factors	2 »
Milker's nodules	1 case

X-ray treatment	1 case
Picture suggestive of acute rheumatism	7 cases

Thus in 35 cases altogether we have some, although in part highly doubtful, indications of a particular etiology. As regards the two salvarsan-treated cases it cannot be decided whether the original disease, the treatment or some entirely different factors have played the principal rôle.

E. multiforme occurring in conjunction with *milker's nodules* has been earlier described by Hallén. Our patient was infected by a cow suffering from cowpox, and the typical exanthema appeared ten days later. In two cases *E. multiforme* occurred in patients suffering from cancer (cancer uteri and Bowen's disease respectively).

In the group of 55 patients with *combined E. multiforme and E. nodosum* there were found, as before stated, in 26 cases indications of a connection with tuberculous primary infection, and in a further 3 cases more uncertain evidence of other connection with tuberculosis. In 3 cases streptococcal infection was noted, in 2 cases the exanthema arose in conjunction with sulphonamide medication, in one case during lobar pneumonia, while in one case it was associated with non-specific positive sero-reactions for syphilis (cf. Löfgren). Eight cases presented a more or less typical picture of acute rheumatism, without signs of tuberculosis or of streptococcal infection. (N. B. This latter point has been insufficiently investigated.) Thus it is only in 11 out of the 55 cases that we are entirely without any data with respect to the etiology.

In this connection I shall briefly mention the group of 17 patients who had *atypical E. multiforme* and where the diagnosis must be designated as doubtful. In one case there were found signs of tuberculous primary infection, in 5 cases streptococcal affections, in one case non-specific positive sero-reactions for syphilis, in one case sulphonamide medication had been employed, while 5 cases presented the picture of polyarthritis, one of them with transition to Bechterew's disease, and, finally, there was one case in which the patient was suffering from chronic polyarthritis.

Discussion.

While it must be kept clearly in mind that many of the etiological possibilities suggested above involve several elements of un-

certainly, yet we cannot fail to note how the first group, with manifestations from mucous membranes, also in this respect stands in striking opposition to the group with combination of E. m. and E. n. In the former group 12 patients out of 65 furnished some etiological data, in the latter group 44 out of 55. Taking the latter group first, it seems, with respect both to sex and age distribution and to the occurrence of tuberculous infection, to show features completely analogous with those of the pure erythema nodosum. In this group we find, besides the tuberculosis, quite the same etiological possibilities as we reckon with in cases of E. nodosum. It is therefore reasonable to suppose that the same etiological factors and perhaps the same pathogenetic mechanism come into play in E. multiforme, accompanied by some few tender nodules on the legs, as in the typical E. nodosum. In other words, that we have here a form of allergic reaction, chiefly to a number of infecting agents, of which the most important are the tubercle bacillus and the hemolytic streptococci. It is likewise probable that the action of the sulphonamides in this connection is of provocative nature, also in cases of E. multiforme, as shown by Löfgren, and further that in some cases it may be a question of a more complicated genesis, possibly a multiple infection, as maintained by Westergren and Löfgren. The justification for regarding, as Löfgren does, cases with non-specific positive sero-reactions for syphilis as being due to a specific etiological factor may seem doubtful, inasmuch as it has been found that various infections of the air passages not infrequently present such reactions (Rein and Elsberg, Vogelsang and others).

The group with affections of the mucous membranes (excepting patients with isolated affection of the eyes) differs in essential points from the group with combined E. m. and E. n. Here there are equally many male and female patients, the frequency of recurrence is 40 per cent, as against only 2 per cent in the combined group, prodromes are far more seldom seen and only exceptionally include joint pains, and the S. R. figures are in general somewhat lower. As regards the etiology, there has not been found a single undoubted case of tuberculous primary infection and altogether the etiological indications are very few. It seems obvious that in the majority of the cases we must look elsewhere for an explanation of the etiology.

There then naturally arises the question of the possibility of *virus infection*. We find quite good indirect support for such an

assumption. The exanthema bears resemblance to varicella, the eruption in the mouth is suggestive of foot-and-mouth disease, for which it may be mistaken. The aphthous stomatitis without *E. multiforme* cannot easily be distinguished clinically from the form which accompanies the exanthema, and is by many supposed to be due to infection by the herpes simplex virus. Herpes around the mouth is not infrequently seen in cases of *E. multiforme*, both with and without simultaneous stomatitis. According to Jersild several authors have noted an accumulation of cases of *E. multiforme* in certain seasons of the year, simultaneously with zoster. In one case in our material zoster occurred in the course of the disease, in another case there was seen lymphocytary choriomeningitis, which may, as we know, be occasioned by virus infection. The strongest support for the assumption that *E. multiforme* with manifestations from mucous membranes may be produced by virus infection is to be found in the investigations respecting the correlation between *E. m.* and primary atypical pneumonia which have been published by Björn Knutsen, by the Fort Bragg Commission on Acute Respiratory Disease, and others. It must be said to be clearly established that the virus which is the cause of the primary atypical pneumonia can also give rise to typical *E. multiforme*.

The fact that it has hitherto not been possible through inoculation and cultivation experiments to detect any virus in cases of *E. m.* is of no great significance. The experiments are few in number, and perhaps the proper method of investigation has yet not been attained.

For the present it seems reasonable to assume that the great majority of the cases of *E. multiforme* with mucous membrane manifestations are caused by a virus, or possibly by several viruses. As regards the pathogenetic mechanism nothing can be said with certainty. That it should be due to a single, specific virus is not very probable. If such is the case, the virus may be the same as that which occasions primary atypical pneumonia. The exudative character of the disease might be taken to point in the direction of an allergic reaction, which might be supposed to be called forth by different viruses. The pathogenesis may also be of more complicated nature. It is difficult to explain the frequent cases of recurrence, although it seems most natural to assume the presence of a chronic focus, from which the reaction is constantly being evoked.

The question then remains whether E. multiforme with manifestations from mucous membranes can be evoked by other infections or toxic agencies than virus infection. As regards lumina

intoxication this must be said to have been established, and as regards the effects of sulphonomamide it must be deemed probable, while the material also embraces some few cases in which streptococcal or staphylococcal infection was present. One can, of course, imagine a collaboration in one or other manner between a virus infection and other infections or toxic factors, analogous with the multiple infection in tuberculosis. I have recently observed a case of primary atypical pneumonia together with typical tuberculous primary complex in a 6-year-old girl, where such collaboration might be supposed to exist.

Remarkable, however, is the negative relation of this group to tuberculosis and to affections of the joints. If other factors than virus infection have any influence in respect to the etiology, that influence seems at all events to be quite inconspicuous.

Finally, we come to the »intermediate group», the cases of E. m. without symptoms from mucous membranes and without nodules due to E. nodosum on the legs. It has already been shown that this group seems in every respect to occupy an intermediate position between the mucous membrane cases and the case with combined E. m. and E. n. The relationship with the latter group is made clear by, *inter alia*, the occurrence of tuberculous primary infection, as an etiological factor and of streptococcal and staphylococcal infections, by the effects of sulphonomamide and by clinical pictures suggestive of acute rheumatism. It is reasonable to assume that a number of cases of the entirely unmixed E. multiforme are etiologically and pathogenetically to be put on a line with the cases in which there also appear some isolated E. nod. efflorescences. We must then include all transitions from the unmixed E. multiforme, through cases with some few tender nodules on the legs, cases with a uniform mixture of typical E. m. and E. n., cases with dominant E. n. and some typical E. m. efflorescences on the upper extremities, face and neck, up to the pure E. nodosum. All these forms are to be regarded as forming an etiological and pathogenetic unit, an allergic mode of reaction to a number of different injurious agents.

On the other hand, we find in the group with pure E. multiforme a far larger number of cases with unexplained etiology than in the »combined» group, and various circumstances seem to in-

dicating that the intermediate group also is related to the cases with mucous membrane manifestations. In the first place, we may find pure E. multiforme with the severest forms of bullous exanthema, which we practically never see in cases where the eruption also embraces E. nodosum nodules. These cases in clinical respects entirely resemble the cases in the mucous membrane group. Further we may find that the patients with pure E. multiforme without mucous membrane affections also show aphthous stomatitis in a subsequent recurrence. I have twice seen pure E. m. in immediate conjunction with *vaccination against smallpox*, and, as already mentioned, the material embraces a case in which the exanthema appeared in conjunction with *milker's nodules*, a disease which must be supposed to be caused by cowpox virus.

It is probable that the cases with pure E. multiforme etiologically and pathogenetically belong partly to the group with mucous membrane affection and must be supposed to be mainly due to virus infections, partly to the group with combined E. m. and E. n. We should then in cases of E. multiforme have to reckon on the whole with two different syndromes, one of which is most often, although not always, complicated by manifestations from mucous membranes and is mainly due to virus infection, while the other is in most (but not all) cases admixed with E. nodosum nodules and represents a mode of allergic reaction to different noxae, whereof the tubercle bacillus and the streptococci seem to be the most important.

Conclusion.

The typical clinical picture of Erythema exudativum multiforme occurs in three main forms, in one of which it appears in combination with affection of one or more mucous membranes, chiefly that of the mouth, in the second as pure E. multiforme, without such mucous membrane symptoms, and in the third as E. multiforme combined with more or fewer E. nodosum nodules on the legs. The line of transition from this latter group to pure E. nodosum is not clearly defined.

The first type, which appears with equal frequency in males and females, is presumably chiefly ascribable to virus infections, but may in exceptional cases also be caused by other toxic or infectious agents. The third type, which is mostly seen in female patients, is pathogenetically and etiologically entirely comparable

with the typical E. nodosum. The second group presumably consists partly of cases of the same type as those which show mucous membrane symptoms and are due to virus infection, partly of cases belonging to the E. nodosum group. Thus we have to reckon with two different syndromes, one of which is most often ascribable to virus infection and is mostly, though not always, attended by mucous membrane manifestations, while the other is in most cases, but not always, admixed with E. nodosum nodules, is etiologically and pathogenetically identical with E. nodosum and represents a form of allergic reaction to various noxae.

La modification du tonus musculaire dans la dystrophie musculaire progressive et son traitement par la malariathérapie.

Par

ALEXANDER ROTTMANN.¹

(Ce travail est parvenu à la rédaction le 29 Septembre 1947.)

Le problème souvent discuté de la pathogénie de la dystrophie musculaire progressive a été bien influencé par les recherches de l'école japonaise de Ken Kuré. En vertu de leur expérimentation animale et de leurs constatations anatomo-pathologiques, ces auteurs ont trouvé que l'explication du complexe pathogénique réside en dernier lieu dans une affection du système neuro-végétatif périphérique. A l'appui de cette opinion j'ai réussi dans des travaux antérieurs à démontrer par la méthode de Minor un trouble de la sudation, et je pouvais ainsi enrichir nos méthodes de diagnostic objectives de cette maladie d'un nouveau procédé concluant.

L'examen histologique de la peau effectué sur des parties présentant des modifications dyshydratiques cliniquement probantes permettait de découvrir aux endroits hyperhydratiques des glandes sudorifères presque hypertrophiées, tandis que les parties anhydrotiques de la peau montraient une dégénérescence des groupes glandulaires.

De plus il y avait dans la plupart des vaisseaux un relâchement du tissu périvasculaire avec augmentation considérable des noyaux adventitiels et infiltration lymphocytaire. Pour des raisons aisées à concevoir nous sommes d'avis qu'il s'agit d'un état d'irritation chronique primitif.

En considération de ces circonstances nous avons tenté une épreuve sur une thérapeutique visant à conditionner l'équilibre

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de l'organisme en activant les forces phsyiques, au lieu de nous reporter à l'administration usuelle de glyocolle et d'adrénaline-pilocarpine. Et cela d'autant plus que Wagner-Jauregg en 1892 pour la singularité du fait a rapporté le cas d'un dystrophique interné à la station neurologique de Graz pour une fièvre typhoïde suivie de la guérison spontanée de la dystrophie musculaire. C'est pourquoi nous avons eu recours à la malariathérapie comme traitement susceptible de donner les meilleurs résultats.

Technique de la malariathérapie:

On administre 5 cm³ de sang d'un paludéen non-syphilitique en injection sous-cutanée. Après 5—8 accès fébriles, suivant compatibilité, on interrompt le traitement par la quinine en doses usuelles. Pendant la réconvalescence on prescrit des fortifiants et des irradiations ultra-violettes. Dès le dixième jour commencement d'une physiothérapie avec exercices gymnastiques en évitant absolument le surménagement et s'adaptant graduellement aux forces augmentantes du malade. Surtout dans les cas invétérés on observe quelquefois immédiatement après la malarisation une faiblesse assez marquée, mais nullement inquiétante, parce qu'elle cède même après une réconvalescence retardée (3—4 semaines) à un remonement général et à une amélioration nette. L'efficacité de la malariathérapie se réalise surtout au commencement de la maladie et pendant les poussées aiguës, tandis que les cas invétérés ne montrent qu'un stationnement avec bien-être subjectif.

L'expérience n'a pas déçu notre attente. Par la malarisation il nous était possible d'influencer favorablement cette maladie décevante aux phases initiales et pendant les poussées progressives. Dans les cas avancés nous avons au moins pu maintenir un état stationnaire. Pour juger de l'amélioration clinique et subjective d'une manière objective il nous semblait judicieux d'examiner les modifications de la créatine survenues par suite de la malariathérapie. Les études de Thomas, Milhorat, Harris et Brand ont porté particulièrement sur le désordre essentiel du métabolisme biochimique du muscle dans la maladie d'Erb, désordre consistant dans l'impuissance du muscle dystrophique de s'assimiler de la créatine (diabète créatinurique).

Par l'administration de glyocolle le taux de la créatine dans le muscle s'accroît, et après une augmentation de la créatinurie au début du traitement celle-ci revient bientôt à la valeur initiale malgré l'application médicamenteuse d'une même quantité. Ce

phénomène fut interprété par Thomas comme une amélioration de la tolérance du muscle à la créatine. D'après cet auteur un trouble primitif du métabolisme de la créatine serait à la base de la dystrophie musculaire progressive; l'application continue de glycocolle devrait donc représenter un effort thérapeutique causal. Mais les nombreuses vérifications ont montré que l'espérance d'une amélioration clinique ne se réalisait pas malgré l'action médicamenteuse sur le biochimisme de l'économie. Si, cependant, l'augmentation de la tolérance à la créatine serait proportionnée à celle de la fonction musculaire croissante, l'argumentation d'une influence de la malariathérapie sur le métabolisme de la créatine semblerait légitime, vu l'efficacité bien évidente de la pyrétothérapie. Or, le métabolisme de la créatine chez les dystrophiques soumis à une malarisation a été étudié par Pichler et Netolitzky de la clinique neurologique de Vienne. Cependant il s'ensuit de leurs recherches que malgré l'amélioration importante après la malariathérapie l'élimination de la créatine reste la même, c'est-à-dire que l'augmentation de la tolérance à la créatine ne peut être considérée comme critère d'une élévation de la fonction du muscle dystrophique. Pour cette raison un nouveau problème s'imposait pour chercher d'autres phénomènes aptes à servir de test objectif de l'amélioration clinique.

L'examen clinique détaillé de nos dystrophiques malarisés montrait chez la plupart des malades le retour des réflexes rotuliens jusque là abolis ainsi qu'une amélioration de la motilité des membres inférieurs. De plus Arnulf Meyer avait observé chez des dystrophiques infantiles malarisés une normalisation du métabolisme basal, dont les valeurs déviaient avant le traitement ou dans le sens positif ou dans le sens négatif. De ce phénomène cet auteur conclut que la malariathérapie exerce une influence essentielle sur l'équilibre physiologique de l'organisme. Aussi avait-il décrit outre la plus grande facilité des mouvements et la disparition des symptômes de fatigue le retour des réflexes rotuliens abolis auparavant. L'étude comparée de l'efficacité d'une cure d'adrénaline-pilocarpine permettait aussi de constater l'amélioration de la motilité et l'augmentation de la réflexibilité; mais après cessation du traitement cette récupération fonctionnelle ne persistait pas. D'autre part il était possible de déclencher les réflexes rotuliens chez les mêmes malades six mois après la malariathérapie.

Toutes ces constatations nous paraissaient indiquer que cette re-

constitution de la réflexibilité comme expression de la modification du tonus musculaire pouvait servir de test objectif de l'effet du traitement. L'examen du tonus des muscles dans la maladie d' Erb était donc dans ces circonstances d'un intérêt capital. Nous avons employé la méthode de Henderson, reposant sur le principe d'un mesurage de la pression interne du muscle produite par un liquide isotone affluant. Au moyen d'un appareil construit par Beigeböck, Junk et Steinlechner, ces auteurs pouvaient confirmer les résultats obtenus par Henderson, à savoir que la valeur normale du tonus musculaire d'un jeune sujet bien portant est généralement de 70 mm H₂O (pression hydrostatique). Ce n'est que vers l'âge de 60 ans que le tonus musculaire baisse approximativement jusqu'à 50 mm. Chez un jeune sujet il faut cependant considérer environ 50 mm comme pathologique. Après des efforts physiques, le tonus musculaire diminue éphémèrement d'environ 20 mm. Des substances agissant sur le sympathique ou le parasympathique, l'adrénaline renforce le tonus musculaire de 25—30 mm; l'acétylholine l'inhibe nettement de 20 mm, tandis que l'atropine et la pilocarpine sont presque sans influence.

Les repérages exécutés chez nos jeunes dystrophiques bien portants du reste, concernant le tonus des muscles dystrophiques des extrémités supérieures et inférieures donnaient des chiffres extrêmement bas, oscillant entre 28 et 38 mm. Ces valeurs se montraient constantes des semaines entières. Il faut surtout relever que ces chiffres se rapportent aussi aux muscles cliniquement non atteints. Au cours des recherches ultérieures nous pouvions observer la persistance du tonus musculaire initial après l'administration de glycocolle, tandis que nous parvenions à renforcer le tonus par la malariathérapie, comme le montrent les observations suivantes:

Obs. I. Malade de 25 ans, repérage des 4 extrémités pris le 20 janvier, avant le traitement: 30, 30, 30, 30.

Examen du 18 mai, pendant la malariathérapie: 28, 42, 35, 38.

Examen du 7 septembre, c. à. d. après 4 mois: 35, 40, 65, 58.

Obs. II. Malade de 28 ans.

Avant le traitement, le 26 janvier: 35, 30, 30, 30.

Le 4 mars, pendant la malariathérapie: 40, 35, 35, 30.

Le 30 mai, 2 mois plus tard: 50, 53, 60, 50.

Le 15 septembre, 4 mois plus tard: 58, 53, 60, 50.

Obs. III. Malade de 12 ans, qui après la malariathérapie montrait les chiffres de 58, 53, 60, 58.

Obs. IV. Malade de 38 ans. Avant le traitement: 38, 38, 40, 38.

4 mois après la malarisation ces chiffres étaient restés les mêmes, de plus il n'y avait pas d'amélioration clinique. Plus tard cependant il s'ensuivit une amélioration évidente et visible dans les chiffres du tonus musculaire: 55, 60, 58, 60.

En considération de ces résultats on voit l'intérêt clinique et thérapeutique de la malarisation. D'un point de vue pathogénique les modifications du tonus du muscle dystrophique accompagnées de troubles du métabolisme basal, de désordres de la sudation et de signes anatomo-pathologiques décrits par Ken Kuré font preuve de la genèse sympathico-végétative de cette maladie.

Summary.

The author reports several cases of dystrophia musculorum progressiva treated by malaria therapy. The important clinical amelioration brought about by this treatment can be measured objectively by the increase of the tonus of the muscle. A comparison between the values found before and after treatment shows a significant elevation of the tonus, and in several cases nearly normal values could be reached.

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Methylthiouracil in the Treatment of Congestive Heart Failure and Angina Pectoris. Results of Prolonged Treatment.

By

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The value of the removal of the influence of the thyroid gland in cases of congestive heart failure and angina pectoris has long been recognized. For that reason Lev and Hamburger (1) and Blumgart, Levine and Berlin (2) introduced total thyroidectomy in the therapy of these conditions. The generally accepted interpretation is that this treatment reduces the total oxygen requirements of the body and thus lessens the load on the heart. More and more evidence has accumulated to prove that removal of the thyroid function alters the response of the cardiovascular system to epinephrine and this alteration is to a considerable extent at least in the cases of angina pectoris, responsible for the improvement (for literature see Raab (3)). The great initial risk of total thyroidectomy in severe cases (4) is the explanation for why this method has not been used more often. The introduction of thiouracil and allied compounds in therapy created possibilities of producing a reversible, riskfree chemical thyroidectomy. It therefore seemed a logical consequence to try treatment with these compounds in cases of chronic congestive heart failure and of angina pectoris. Favourable immediate results from this treatment have also been published (3, 5, 6, 7, 8, 9, 10). In most reports thiouracil or allied derivatives have been administered for rather short periods of time usually less than ten months. This paper reports the results of a continuous long standing methylthiouracil therapy in cases with congestive heart failure and angina pectoris.

Material and Methods.

Since August 1944 we have used continuous treatment with methylthiouracil in seven cases of chronic congestive heart failure and in nine proven cases of angina pectoris. All of the cases with congestive failure had rheumatic valvular heart disease. The criteria used in the diagnosis of angina pectoris have recently been described (11). None of the patients had any signs of hyperthyroidism. The early results of the therapy in some of the patients have been reported in earlier publications (6, 7). Three of the patients have been continuously treated and observed for more than three years, three for more than two years, five for more than one and a half years, five for more than one year and one for more than six months.

The compound used in every case was methylthiouracil. Because of sensibilisation to this drug the treatment was later changed to thiouracil in one instance. The initial dose was 400—500 mg a day divided into doses of 100 mg. This system of dosing was continued until the patients were discharged from the hospital or when clinical improvement during the stay in the hospital occurred. The dose was then reduced to 100 mg three times daily and this dose was continued until the basal metabolic rate was lowered or a significant improvement had occurred. The dose was then further decreased to 100 or 200 mg a day and was then adjusted according to clinical improvement and the basal metabolism. Thus, in some cases where a slight myxedema occurred, it was in the subsequent course easy to maintain the metabolic rate at a desired level with very small daily doses (15 to 50 mg). The dose was therefore adjusted from case to case.

Most of the patients have been treated and stayed in the hospital for several months before discharge and have then been kept under continuous and regular control every month or every second month. It is obvious that improvement of the treatment is easier to evaluate in chronic congestive heart failure than in angina pectoris. In angina, much of the disturbance is subjective and we have therefore tried to record objective signs of improvement. In every case of angina, functional tests, hypoxemia and exercise tolerance tests, were done before the treatment was started, and then as a rule at regular intervals during therapy. Hypoxemia tests were done with 10 per cent oxygen in 90 per

cent nitrogen until typical anginal pain occurred; otherwise the test was discontinued after 20 minutes. Electrocardiograms were taken before the test, every 5 minutes during it, at the time the patient noted pain, and at the end of the test. The effect upon pulse and blood pressure were recorded and the oxygen saturation of the blood was followed with the oximeter (12). Levy's (13) criteria for a pathological test have been used.

Exercise tolerance was tested with Krogh's bicycle ergometer with increasing load and simultaneously pulse and EKG were registered. The test was interrupted when typical pain occurred. The test was started with a light load (300 kgm/min.). After 6 minutes' work with this load, and if no discomfort or pathological pulse reaction occurred, the load was increased to 600 kgm/min. for another 6 min. If this work provoked no side reactions the load was increased to 900 kgm/min. for 6 minutes and if the patients tolerated this exertion a work of 1,200 kgm per min. was performed. In the evaluation of these tests besides occurrence of typical pain and the behaviour or the pulse rate attention was paid to changes in the EKG. Further details concerning the hypoxemia and exercise tolerance tests have already been published (11). In the following only the figures of the total amount of work (in kgm) done in the different exercise tests are given. Changes in the EKG during rest which indicate coronary insufficiency have also not been specified and such EKG changes have been called coronary insufficiency. The functional capacity of the patients has been classified according to the criteria committee of the New York Heart Association (14). The material has been divided into two groups, one group comprising the cases of congestive heart failure and in one group including the cases with angina pectoris.

Congestive Heart Failure.

Case Reports.

Case 1. M. J. P. (St. Erik's Hospital No. 6359/44), a thirty-five-year old housewife with mitral stenosis diagnosed in 1933. In 1936 and 1937 attacks of pulmonary edema. Since that time gradually increasing shortness of breath and palpitation, after exertion she often had mild attacks of pulmonary edema or episodes with transitory auricular fibrillation. These symptoms became accentuated in the summer of 1944 and it was then difficult to keep her in a fully compensated state. In connection with an attack of auricular fib-

rillation she was admitted to the hospital, October 30, 1944. The next day regular rhythm was restored. Physical examination revealed a presystolic murmur at the apex, basal pulmonary râles and the blood pressure was 120/80. Otherwise physical examination was negative. The chest X-ray showed slight enlargement of the heart, the volume being 530 ml/m² of body surface and pulmonary stasis. EKG showed right axis deviation with broad P waves. The basal metabolic rate was ± 0 per cent, the serum cholesterol 200 mg per cent. Methylthiouracil 100 mg five times daily, was started November 2, 1944. In connection with a severe acute virus pneumonia at the end of February 1945 the drug was temporarily withdrawn between February 28 and March 21. In the middle of May she had improved markedly and the dose was reduced to 100 mg twice daily. June 6, the basal metabolic rate was minus 15 per cent and the serum cholesterol 345 mg per cent. She felt well and had no subjective symptoms from the heart. Chest X-ray showed unchanged size of the heart and a disappearance of the pulmonary stasis. Two months later her basal metabolic rate was minus 24 per cent and the serum cholesterol 342 mg per cent. The dose of methylthiouracil was reduced to 75 mg a day but in spite of this she developed a slight myxedema three weeks later September 9, 1945 the basal metabolic rate was minus 28 per cent and the serum cholesterol 492 mg per cent. The drug was then discontinued for 30 days. At that time her shortness of breath had practically disappeared and she could climb upstairs without difficulty. Since that time her maintenance dose of methylthiouracil has varied between 25 and 100 mg a day. With this dose her basal metabolic rate has been between minus 6 and minus 23 per cent, usually being around minus 15 per cent. Since June, 1945 she has felt well and has had no symptoms from her heart and has not had a single attack of pulmonary edema or auricular fibrillation. Her functional capacity has improved, she now runs a big house without discomfort and has adopted two small children whom she is bringing up alone. The size of the heart was unchanged June, 13, 1947 and the EKG November 17, 1947 showed normal rhythm, 68 beats per minute. She has continuously been given a refractory dose of digitalis since 1936.

Comment.

The functional capacity of this patient with mitral stenosis corresponds to Class III. After six and a half months' methylthiouracil treatment she showed a marked improvement and after eight and a half months' therapy her condition belonged to Class I. This improvement was coincident with a drop in the basal metabolism. After eight and a half months' therapy this case developed a slight myxedema. With small doses of methylthiouracil her condition since then has remained unchanged for more than two years.

Case 2. S. B. J. (St. Erik's Hospital No. 7080/44), a forty-seven-year old housewife with mitral stenosis since 1909. With a refractory dose of digitalis and a restricted life she remained in a fairly good condition until the beginning of 1940. Since that time she has been constantly decompensated with repeated attacks of pulmonary edema. Since January, 1944 she has been practically hospitalized the whole time and besides frequent milder episodes she has had eight alarming attacks of pulmonary edema which necessitated emergency hospital care. She was admitted to the hospital in a state of fully developed pulmonary edema December 2, 1944. With ordinary measures the edema disappeared. The examination disclosed an auricular fibrillation, a systolic and a diastolic murmur with maximum intensity at the apex and a blood pressure of 185/90. X-ray study showed an enlargement of the heart, the volume being 610 ml/m² of body surface. The basal metabolic rate was plus 16 per cent and the serum cholesterol 213 mg per cent. Methylthiouracil, 100 mg five times daily, was started December 15, 1944. In April 1945 she had improved markedly and had been free from attacks of pulmonary edema for three weeks. The patient then failed to return for further observation until she was admitted to the hospital with a severe attack of pulmonary edema, September 26, 1945. The reason why she had escaped control was that she felt so well and thought observation was not necessary, and she had stopped the drug on her own accord at the end of May. The size of the heart had increased to 810 ml/m² of body surface. Methylthiouracil was again started, September 28, and she was dismissed from the hospital December 21, 1945. Because of slight attacks of pulmonary edema she was readmitted to the hospital January 19, 1946. X-ray study showed unchanged volume of the heart, 840 ml/m² of body surface and she had a persistent auricular fibrillation. In the beginning of March she improved, March 7, 1946 her basal metabolic rate was minus 13 per cent and her serum cholesterol 326 mg per cent. At the same time the volume of the heart had decreased to 690 ml/m² of body surface. Since that time she has been in a good condition with gradually diminishing doses of methylthiouracil. For more than 20 months she has not had a single attack of pulmonary edema and has been able to run her home without much discomfort. The basal metabolism has not been lowered to any considerable extent and has varied between plus 6 and minus 15 per cent. The serum cholesterol has been slightly elevated since March, 1946, around 300 mg per cent, indicating the blocking effect of methylthiouracil upon the synthesis of thyroxin. She has still a persistent auricular fibrillation.

Comment.

This patient with mitral stenosis had been decompensated for four years and had practically been confined to bed during the last ten months. A characteristic feature of her condition was the frequent often serious attacks of pulmonary edema. Her func-

tional capacity thus belongs to Class IV. After four months' treatment with methylthiouracil she improved markedly and because of this she failed to return for further controls and stopped treatment on her own accord after five and a half months of therapy. Three months later symptoms returned and she was again hospitalized for a severe attack of pulmonary edema. Methylthiouracil treatment was started and after five and a half months a marked improvement again occurred. She has since then for more than 20 months, been in a good condition and completely free from attacks of pulmonary edema. The functional capacity of the heart has also improved and she now belongs to Class II.

Case 3. E. R. H. (St. Erik's Hospital No. 6190/44), a thirty-seven-year old male headwaiter, with mitral stenosis. Since August, 1943 he had had persistent auricular fibrillation and decompensation. He was admitted to the hospital October, 23, 1944. Examination showed a rapid auricular fibrillation, a systolic and diastolic murmur with maximum intensity over apex, a blood pressure of 140/100 and slight enlargement of the liver. Chest X-ray showed a large heart, the volume being 850 ml/m² of body surface. EKG showed auricular fibrillation alternating with auricular flutter with changing blocking 2:1 to 4:1. On repeated controls he usually had an auricular flutter. After digitalization a trial with quinidine was made with a negative result. The basal metabolic rate was minus 6 per cent and the serum cholesterol 156 mg per cent. Methylthiouracil, 100 mg five times daily, was started November 11, 1944. After four months' therapy he had improved and a new trial to restore the rhythm with quinidine was made, without result. He then improved still further and in June 1945 he had no subjective symptoms from his heart. June 15, the basal metabolic rate was minus 15 per cent and the serum cholesterol 312 mg per cent. The volume of the heart was 660 ml/m² of body surface and he had a slow auricular fibrillation. In spite of diminished doses of methylthiouracil he developed two months later a slight myxedema (B. M. R. being minus 18 per cent, serum cholesterol 342 mg per cent). At that time, August 24, he had regular rhythm and no symptoms at all from the heart. He felt well and had a regular rhythm until the beginning of June 1946. The month before he had been working hard and had taken his medicine irregularly. Symptoms gradually reappeared, he complained of increasing shortness of breath and his heart action became irregular. Simultaneously with this deterioration the basal metabolic rate, usually around minus 10 per cent, had risen to plus 12 per cent. He was hospitalized again July 25, 1946. X-ray study showed that the heart had increased in size, the volume being 830 ml/m² of body surface and the EKG showed auricular fibrillation or auricular flutter. The heart action has never since been regular. His dose of methylthiouracil was increased and at the end of August his basal

metabolism had dropped to minus 20 per cent and has been kept around this level. He improved rapidly and six weeks later he was discharged from the hospital. He was then in a fairly good condition for another three months after which he became worse again and had a slight attack of pulmonary edema. This episode subsided rapidly and he has later on two occasions been decompensated. At present his functional capacity is fairly good. He still has irregular heart action.

Comment.

This patient with mitral stenosis and auricular fibrillation and flutter has had an increasing decompensation for more than one year and had showed no particular response to usual therapy. His functional capacity belongs to Class IV. After four months' therapy with methylthiouracil he improved and after nine months treatment the heart rhythm became spontaneously regular. At that time he was fully compensated and had no subjective symptoms from his heart. This improvement occurred in conjunction with a fall in the basal metabolic rate. He then remained in the same condition with regular rhythm for another nine months. At that time he was temporarily decompensated and the heart became irregular. He has since been in fairly good condition during the further 20 months he has been under observation. During that time he has been decompensated on three occasions and he has the whole time had persistent irregular rhythm of the heart. His functional capacity now belongs to Class III.

Case 4. E. A. S. (St. Erik's Hospital No. 243/45), a sixty-five-year old female office clerk, with mitral stenosis. With a refractory dose of digitalis she had been in fairly good condition until the last five years when she often got transitory attacks of auricular fibrillation and slight decompensation. She was admitted to the hospital suffering from such an attack January 12, 1945. On admission she had auricular fibrillation, a systolic and a diastolic murmur at the left sternal border and a blood pressure of 145/80. X-ray study of the chest showed a heart volume of 845 ml/m² of body surface and the EKG showed auricular fibrillation. With ordinary measures her heart rhythm was restored to normal after one month's hospitalization. After the discharge she was fairly well but tended to develop attacks of auricular fibrillation on exertion. Methylthiouracil treatment, 100 mg four times daily, was therefore started November 26, 1945. Her basal metabolic rate before this treatment was plus 1 per cent and the serum cholesterol 259 per cent. After three and a half months' therapy she felt better and during the month before she had no attacks of auricular fibrillation. She then remained in good condition

and had no attacks of auricular fibrillation for another ten months. Her basal metabolic rate during this time varied between plus 2 and minus 6 per cent and her serum cholesterol was usually around 300 mg per cent. At the end of December, 1946 she had an attack of cholecystitis and in connection with this the heart became irregular. The heart has since been fibrillating and an attempt to restore the rhythm during one month's stay in the hospital in the beginning of 1947 was unsuccessful. In the middle of March 1947 the drug was discontinued. She is now in fairly good condition.

Comment.

This case with mitral stenosis had had increasingly frequent attacks of auricular fibrillation during the last five years. She belonged to Class III. After three months' therapy she improved and was free from subjective symptoms and attacks of auricular fibrillation for eleven months. The B. M. R. was not lowered to any considerable extent during this time but there was a slight rise in the serum cholesterol. In connection with an acute attack of cholecystitis she started to fibrillate again and her heart action has since been irregular. The drug was discontinued after sixteen months of therapy and she is still eight months after the withdrawal of methylthiouracil, in fairly good condition, corresponding to Class II.

Case 5. G. A. N. (St. Erik's Hospital No. 7473/45), a forty-one-year old taxidriver with mitral stenosis. Since 1943 auricular fibrillation and increasingly severe decompensation. Admitted to the hospital December, 5, 1945. Examination disclosed auricular fibrillation, a systolic and diastolic murmur with maximum intensity over apex, a slightly enlarged liver and a blood pressure of 110/65. Chest X-ray showed an enlarged heart, the volume being 825 ml/m² of body surface and EKG showed auricular fibrillation. B. M. R. was plus 9 per cent. Methylthiouracil, 100 mg five times daily, was started December 14, 1946. He improved gradually and when he was discharged from the hospital four months later, April 6, 1947 he was fully compensated and felt well. He started to work as a headwaiter two months later and had no subjective symptoms from his heart. The condition remained unchanged for another five months. During this period there was no marked change in the B. M. R., it varied between plus 11 and minus 8 per cent. In the beginning of December, *i. e.* after one year's therapy, he got fever and a rash which immediately subsided when the drug was withdrawn. Test doses of methylthiouracil and thiouracil proved that he had become sensitized to these drugs and further treatment with these compounds was stopped, December 13, 1946. One month later he had serious and repeated attacks of pulmonary embolism and he became decompensated with pronounced

peripheral edema. His condition improved very slowly and he is now in a condition corresponding to Class III. The whole time he has had persistent auricular fibrillation.

Comment.

The functional capacity of this patient belonged to Class III or IV before treatment. He improved considerably after four months' therapy and was able to start work again. This improvement lasted for another five months when the therapy was stopped because of sensitisation for thiouracil drugs. His condition now, eleven months after withdrawal of this treatment, is about the same as on admission.

Case 6. E. V. K. P. (St. Erik's Hospital No. 1847/45), a fifty-two-year old retired taxidriver with mitral stenosis. He had had a persistent auricular fibrillation since 1941 and since 1943 he had been unable to work because of his heart failure. During the last three years he had been hospitalized on several occasions and he responded less and less to ordinary therapeutic measures (bedrest, digitalis and mercurial diuretics). He was admitted to the hospital March 5, 1946. On admission he was severely decompensated, with pronounced dyspnea, a rapid auricular fibrillation and basal pulmonary râles. A systolic and diastolic murmur with maximum intensity over apex was heard and the blood pressure was 130/75. X-ray study showed an enlargement of the heart, the volume being 860 ml/m² of body surface and pulmonary stasis. Methylthiouracil, 100 mg five times daily, was started March 6, 1946. The B. M. R. before this treatment was plus 6 per cent and the serum cholesterol 256 mg per cent. He stayed in the hospital for six months and was at that time considerably improved. The basal metabolic rate was then minus 12 per cent and the serum cholesterol was 311 mg per cent. The dose of methylthiouracil was decreased and he was in fairly good condition. After 12 months' therapy he had a slight myxedema (B. M. R. being minus 18 per cent, serum cholesterol 372 mg per cent). At that time he was in a compensated state. With diminishing doses the myxedema soon disappeared and he remained in fairly good condition until June 1946 when the previous symptoms gradually returned. The B. M. R., which was usually around minus 10 per cent had at that time risen to plus 2 per cent. He was again admitted to hospital July, 2, 1947 and as he did not show any further improvement on methylthiouracil therapy the drug was discontinued August 11, 1947. He is now in a chronic decompensated state.

Comment.

The functional capacity of this patient belonged to Class IV. He improved after six months' treatment and after twelve months' therapy his condition belonged to Class II or III. The improve-

ment was coincident with a drop in the basal metabolism, after 12 months' therapy a slight myxedema occurred. The improvement remained another three months, then he was decompensated again and further methylthiouracil treatment failed to improve his state. He now again belongs to Class IV.

Case 7. H. M. B. (St. Erik's Hospital No. 7362/45), a forty-year old seamstress with mitral stenosis. Because of increasing decompensation she had been confined to bed for the past year and she responded very little to ordinary therapeutic measures. She was admitted to the hospital November 11, 1945. On admission she was severely decompensated, with peripheral edema, liver enlargement, ascites and transudate in both pleurae. She had a regular rhythm and a systolic and diastolic murmur with maximal intensity over apex was heard. The blood pressure was 115/85. Chest X-ray showed an enlarged heart, the volume being 780 ml/m² of body surface. Her basal metabolic rate was plus 31 per cent and her serum cholesterol 220 mg per cent. Methylthiouracil, 100 mg five times daily was started December 2, 1945. After two months' therapy she developed moderate leukopenia with a normal differential count and the drug was therefore discontinued for five days. The white blood cells rapidly returned to normal values and the treatment was started again. After four months' therapy she again had a drop in the white cells and the drug was again stopped for five days. At that time she had improved, her shortness of breath was considerably improved and she was able to walk around in the hospital garden. We therefore thought it worth while to continue with methylthiouracil. Six weeks later she again developed a marked leukopenia, 1,900 white cells, and the drug was therefore stopped June 7, 1946. She was at that time in fairly good condition. After the withdrawal of the drug, symptoms of pronounced decompensation rapidly returned and she died June 29, 1946. During the methylthiouracil therapy her B. M. R. was never lowered and showed variations between plus 29 and minus 4 per cent.

Comment. ☞

This patient belonged to Class IV. During methylthiouracil therapy she improved somewhat after four months' treatment. Because of toxic effects the drug was discontinued after six months' therapy. Thereafter symptoms rapidly returned and she died one month later.

These seven cases with mitral stenosis and congestive heart failure were all severe, four of them belonged to Class IV and three to Class III before methylthiouracil treatment was started. They had previously shown little or no response to usual therapy

(bed-rest, digitalis and mercurial diuretics). Four patients showed a pronounced and the remaining three patients a moderate improvement when ordinary therapy was supplemented with methythiouracil. Thus patients 1 and 2 improved considerably and have then, with continuous treatment, remained in this condition for two years. Case 3 who had had a therapy-resistant auricular fibrillation for a long time became without further therapeutic measures, spontaneously regular and the rhythm was then normal for another nine months. Of the cases with moderate or transitory improvement therapy was stopped because of toxic effects in cases 5 and 7. After the withdrawal of the drug these patients rapidly returned to the condition they were in before treatment. Case 6 had shown an improvement over a period of six months when in spite of continuous treatment, the previous symptoms gradually reappeared. Further therapy for fourteen months has failed to improve his condition. With the dose of methythiouracil we have used, improvement in cases of congestive heart failure was not evident until after four to six months' therapy. In cases 1, 3 and 6 the improvement coincided with a drop in the basal metabolism; all these patients developed a slight myxedema after eight to twelve months' treatment. In the further course a basal metabolic rate of about minus 15 per cent was aimed at. In the remaining cases there was no more pronounced lowering of the basal metabolism. There was, however, a rise in the serumcholesterol of between 300 to 350 mg per cent indicating the blocking effect of the drug upon the synthesis of thyroxin.

Angina Pectoris.

Case Reports.

Case 1. G. A. H. (St. Erik's Hospital No. 6418/45), a fifty-four-year old male musician, oboist, had had occasional attacks of chest tightness and pain for 9 years. Since the beginning of 1945 these attacks became increasingly more frequent and during the month previous to his admission to the hospital he had frequent attacks every day, sometimes every hour or every second hour, and they were no longer adequately controlled by nitroglycerin. The slightest exertion provoked an attack. He must stop his work and was closely confined to his home. On admission October 22, 1945 the physical examination revealed no distinctive abnormalities. The blood pressure was 160/90, the heart size was normal and a faint systolic murmur was heard over the apex. The EKG showed coronary insufficiency. Hypoxemia test October 31, was positive, after 15 minutes 6—483329. *Acta med. Scandinav.* Vol. CXXXII.



Fig. 1 a.

Fig. 1 b.

Fig. 1. a) EKG before the test.

b) EKG after 20 min. test (oxygen saturation of blood 67 per cent).

he got severe pain and the changes in EKG was markedly increased (fig. 1). At an exercise tolerance test, November 2, the total amount of work he could do was 960 kgm before he got pain and pronounced changes in the EKG.

The initial basal metabolism was plus 30 per cent and the serum cholesterol 193 mg per cent. Methylthiouracil, 100 mg five times daily, was started November 5, 1945. After six weeks therapy he commenced to improve and after four months' therapy he had attacks of pain very seldom. His basal metabolic rate at that time, March 26, 1946, was minus 7 per cent and the serum cholesterol 312 mg per cent. The exercise tolerance test was unchanged; the hypoxemia test could be performed for 20 minutes with only slight pain, but the changes in the EKG were about the same as before treatment. He then further improved and started to work as an oboist in a symphony orchestra in the middle of May 1946. He has since *i. e.* for more than 20 months, with decreasing doses of methylthiouracil, been practically free from symptoms and been able to do his work without difficulty. The basal metabolism has varied between ± 0 and minus 16 per cent, usually being around minus 10 per cent. Hypoxemia test August 31, 1946, showed a marked improvement. He performed 20 minutes without any pain and the changes in the EKG were small (fig. 2). The exercise tolerance had at the same time increased fourfold, he could do a work of 4,320 kgm without pain, but this effort provoked EKG changes.

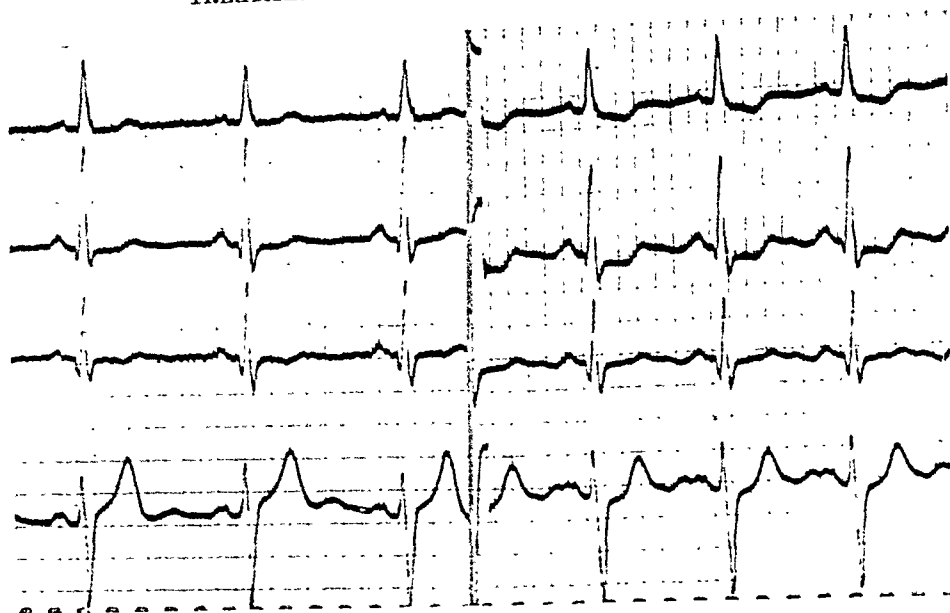


Fig. 2 a.

Fig. 2 b.

Fig. 2. a) EKG before the test.

b) EKG after 20 min. test (oxygen saturation of blood 56 per cent).

Comment.

This case of severe angina pectoris belonged to Class IV. He improved considerably after six weeks treatment and after four months' therapy he was practically free from subjective symptoms and was able to take up his work again. Objectively, there was an improvement in the hypoxemia and exercise tests. The improvement was coincident with a drop in the basal metabolism. He has remained in this condition during the further 20 months he has been observed. He now belongs to Class II.

Case 2. E. A. (St. Erik's Hospital No. 2729/45), a fifty-one-year old ironmonger, had for four years had precordial tightness and pain brought on by exertion, emotional disturbance and exposure to cold. For the last ten months preceding hospitalization the attacks of pain had become more frequent and severe, once or twice a day. They were controlled with nitroglycerin. He was admitted to the hospital September 19, 1945. Examination revealed nothing particular, the blood pressure was 160/100. X-ray study of the chest showed a slight enlargement of the heart, volume being 610 ml/m² of body surface. EKG showed left axis deviation. The hypoxemia test had to be interrupted after 13 minutes because of pain, and the EKG showed marked changes. At an exercise tolerance test a total amount of work of 1,800 kgm could be done. The basal metabolic rate was minus 6 per

cent, serum cholesterol 205 mg per cent. Methylthiouracil, 100 mg five times daily, was instituted September 30, 1945. After two months' therapy he had improved markedly and had no subjective symptoms. The basal metabolism and the dose of methylthiouracil was therefore diminished to 200 mg a day. He has since that time, for more than 23 months been in excellent condition and been working hard and started hunting again. Only after strenuous exertion he gets light attacks of chest tightness. Hypoxemia tests April 8, 1945 and March 8, 1947 were interrupted because of pain after respectively 19 and 15 minutes. The EKG changes were lesser than before treatment. The exercise tolerance has improved about seven times, the total amount of work he could do without pain was 4,200 in September 20, 1946 and 10,800 kgm in March 8, 1947. The B. M. R. has during this time varied between plus 15 and minus 10 per cent.

Comment.

This patient with moderately severe angina pectoris belongs to Class II or III. He improved considerably after two months' therapy, and has remained symptomfree during the subsequent 23 months under observation. His hypoxemia and exercise tolerance tests have improved markedly during treatment. No significant lowering of the basal metabolic rate has occurred during the therapy.

Case 3. A. E. A., a sixty-one-year old lawyer had for four years had increasingly frequent attacks of chest tightness and pain radiating down the left arm. For the last two years exhaustion followed almost any kind of exertion or any kind of emotional stress. For instance, he could just walk one or two steps before he had an attack and following a meal he almost regularly developed chest pains. He was therefore closely confined to his home. The physical examination revealed no distinctive abnormalities. The blood pressure was 160/110, the heart size was normal and there were no murmurs. The EKG showed coronary insufficiency. The hypoxemia test, because of severe pain, had to be interrupted after five minutes and the EKG showed markedly increased changes. The basal metabolic rate was minus 10 per cent. Methylthiouracil, 100 mg five times daily, was started October 6, 1945. In April 1946 the B. M. R. had dropped to minus 20 per cent without any noticeable improvement of his condition. In the middle of May 1946 he discontinued the drug on his own accord and escaped further controls until January 10, 1947. His condition was at that time, on the whole, the same as on his first visit. Methylthiouracil, 100 mg three times daily, was started again. His condition since then improved very little and he died of myocardial infarction December 15, 1947.

Comment.

In this case of severe angina pectoris, belonging to Class IV, methylthiouracil administered for seven and a half months failed

to produce any noticeable improvement and the patient then stopped the drug on his own accord. Six months later methylthiouracil was instituted again. After continuous therapy for eleven months no significant improvement occurred.

Case 4. A. C. K. (St. Erik's Hospital No. 132/46), a seventy-five-year old housewife, had recovered from an acute myocardial infarction 1944, which had been preceded by several years of precordial tightness and pain radiating down both arms brought on by exertion. For the last six months she had been having frequent attacks of pain with shortness of breath even after slight exertion. She was admitted to the hospital January 5, 1946. Physical examination was negative, the blood pressure was 110/70 and no heart murmurs were heard. Chest X-ray showed a heart of normal size, volume being 340 ml/m² of body surface. EKG showed no distinctive abnormalities. A hypoxemia test could be performed for 20 minutes, with slight pains, and no significant changes in the EKG occurred. The basal metabolic rate was minus 12 per cent. Methylthiouracil, 100 mg four times daily, was started January 9, 1946. After two months she had improved considerably and after five months' treatment she had practically no subjective heart symptoms at all. At that time she had a slight myxedema. B. M. R. being minus 25 per cent and serum cholesterol 503 mg per cent. The dose of methylthiouracil has since then been gradually diminished and her basal metabolism has been varying between minus 4 and minus 25 per cent usually being around minus 15 per cent. Up till now she has remained practically symptom-free for 19 months. She could perform a hypoxemia test August 23, 1946, without any pain or EKG changes.

Comment.

This case of moderately severe angina belonged to Class III. She showed an improvement after two months' therapy and after five months' treatment she became symptomfree. The improvement was coincident with a fall in the basal metabolism. With small daily doses of methylthiouracil she has remained symptom-free for nineteen months.

Case 5. S. E. M. Ö. (St. Erik's Hospital No. 6551/45) a fifty-one-year old housewife, had been having attacks of precordial pain and distress for six years. These attacks have gradually increased in frequency and during the last year even the slightest exertion precipitated an attack of severe pain radiating into the back and throat and both arms. Because of these intractable pains a cervico-thoracic ganglionectomy including C 8—Th 5 on the left side was performed April 27, 1945. A fortnight later she developed a traumatic neuritis with severe pains only relieved by huge doses of morphine. She became a morphinic and barbituric addict, developed acute psychosis

and was treated for one month at a psychiatric ward. Three months after the operation the symptoms of her angina still persisted, being now mainly rightsided. Because of the unbearable pains she tried to commit suicide with amytal. She was found unconscious in her home October 27, 1945 and was immediately admitted to the hospital. She was unconscious for another three days and was then transported to a psychiatric ward where she was treated until January 1946. The symptoms of angina pectoris were unchanged. At that time the physical examination was negative, the blood pressure was 140/100. Chest X-ray showed no increase in heart size and the EKG left ventricular preponderance. The hypoxemia test had to be interrupted because of pain after 11 minutes and there were very marked EKG changes. The basal metabolic rate was plus 19 per cent. Methylthiouracil, 100 mg five times daily, was started February 17, 1946. After three months' therapy she was much improved and the B. M. R. was then minus 6 per cent. June 12, 1946, she could perform a hypoxemia test for 20 minutes with only slight pain and no changes in the EKG. With decreasing doses of the drug she continued to improve, her B. M. R. was around minus 10 per cent until the beginning of September 1946 when she developed a slight myxedema. At that time she had only occasional mild attacks of angina pectoris and was often completely symptomfree for five to six days. One month later, in connection with severe influenza, she relapsed. The basal metabolic rate had then risen to plus 23 per cent. The dose of methylthiouracil was increased and six weeks later she had improved again. Since the middle of December 1946 she has remained in a fairly good condition and her B. M. R. has varied between plus 5 and minus 14 per cent, usually being around minus 10 per cent.

Comment.

This patient with extremely severe angina pectoris was operated upon with cervico-thoracic ganglionectomy without any effect. Her condition belonged to Class IV. After three months' therapy with methylthiouracil she improved and after seven months' treatment the improvement was considerable. Coincident with the improvement there was a drop in the basal metabolism to a slight myxedema. In connection with influenza the basal metabolic rate rose and she relapsed. With an increased dose of the drug the symptoms were controlled again and she has since for eleven months been in fairly good condition corresponding to Class II. The hypoxemia test became negative during treatment.

Case 6. K. F. S. (St. Erik's Hospital No. 7194/45) a forty-one-year old doorkeeper at a bank had had attacks of precordial pain and distress for five years. In July 1944 these attacks gradually became very frequent and severe and the slightest exertion provoked

an attack. He had had to give up his work and remained closely confined to his home. Because of his intractable angina pectoris bilateral cervico-thoracic ganglionectomy was performed January 11 and 24, 1945. After the operation he improved markedly and could resume his job and was in fairly good condition until September 1945. The attacks of angina then gradually returned. The pain had prior to the operation, radiated into both shoulders and the left arm and was now radiating into the throat, neck and jaw. In January 1946 the attacks of angina pectoris were about as frequent and severe as before the operation. The physical examination at that time revealed no distinctive abnormalities, the blood pressure was 150/95 and no heart murmurs were heard. Chest X-ray showed a heart of normal size and the EKG was normal. The hypoxemia test could be performed for 20 minutes with moderate pain and no EKG changes. At an exercise tolerance test the total amount of work he could do before he got pain was 3,000 kpm. The basal metabolic rate was minus 7 per cent and the serum cholesterol 245 mg per cent. Methylthiouracil 100 mg five times daily, was started March 18, 1946. After five months' therapy he showed some improvement and that was more pronounced one month later. August 8, the hypoxemia test could be performed for 20 minutes with very slight pain and no changes in the EKG. The exercise tolerance test was unchanged. At the end of October the attacks of pain became more frequent again. He remained in this condition until the middle of January 1947 and the basal metabolism during this period varied between plus 16 and minus 12 per cent. January 15, 1947 the hypoxemia test, because of pain had to be interrupted after 10 minutes, no EKG changes occurred. From now on he improved considerably and became free from nocturnal pains, and he has since for nine months remained in fairly good condition and has started to work again. The basal metabolic rate has varied between minus 15 and minus 28 per cent usually being around minus 20 per cent. February 21, 1947 the hypoxemia test could be performed for 20 minutes with moderate pain and no EKG changes. At the same time the total amount of work he could do before he got pain had increased about four times, being 10,800 kpm.

Comment.

This patient was because of intractable angina pectoris operated upon with bilateral cervico-thoracic ganglionectomy. He improved after operation but eight months later symptoms returned and his condition belonged to Class IV when treatment with methylthiouracil was instituted. He improved somewhat after five months' therapy but his symptoms was about as severe as before treatment, three months later. After 10 months' treatment he improved again, and to a considerable extent. This improvement was coincident with a more pronounced and more lasting lowering of the basal metabolism and it has then lasted on the whole un-

changed for a further nine months. There was a marked improvement in the exercise tolerance and he now belongs to Class II or III.

Case 7. J. H. P. (St. Erik's Hospital No. 3412/46) a sixty-five-year old forester, developed chest pain and tightness with shortness of breath on exertion ten months previous to his admission May 16, 1946. These symptoms increased in frequency and severity very rapidly, the attacks would follow each meal and would come on after a walk of five to ten meters. He was unable to work. On admission the examination revealed nothing particular. The blood pressure was 150/105, and a systolic murmur with maximum intensity over the aortic valve was heard. X-ray of the chest showed a heart of normal size and the EKG showed coronary insufficiency. The hypoxemia test was interrupted after 10 minutes because of pain and there were pronounced EKG changes. At an exercise tolerance test the total amount of work he could do was 2,400 kgm. The basal metabolic rate was plus 6 per cent and the serum cholesterol 214 mg per cent. Methylthiouracil, 100 mg five times daily, was started June 11, 1946. After two months' treatment he had improved markedly. July 27, he could perform a hypoxemia test for 20 minutes with slight pains and moderate EKG changes. The exercise tolerance was at the same time 5,400 kgm. When he was discharged from the hospital September 11, 1946 he was symptomfree. With decreasing doses of methylthiouracil he has remained free from symptoms and has been working at full capacity for another fourteen months. The basal metabolic rate has, during the whole time, not been lowered to any considerable degree, varying between plus 13 and minus 6 per cent. The rise in serum cholesterol to around 300 mg per cent shows the influence of the drug. Fifteen months after therapy was started he had clinically a slight myxedema, with a serum cholesterol of 435 mg per cent and a considerable enlargement of the thyroid gland. The basal metabolic rate was plus 9 per cent. With further diminishing doses the myxedema soon subsided. March 25, 1947 the hypoxemia test became negative and the exercise tolerance had increased more than six times, being 15,600 kgm.

Comment.

This patient with moderately severe angina belonged when treatment was started, in Class IV. He improved rapidly and became symptomfree after three months' treatment and then remained free from symptoms for another fourteen months. The hypoxemia test became negative and the exercise tolerance improved markedly during treatment.

Case 8. J. C. W. (St. Erik's Hospital No. 3381/46), a fifty-year-old farmer had had frequent attacks of precordial pain and tightness,

brought on by exertion, emotional distress and following meals. He had had to stop his work as a farmer. On admission to the hospital July 25, 1946 the examination apart from a blood pressure of 175/100, disclosed no distinctive abnormalities. X-ray of the chest showed a slightly enlarged heart, the volume being 500 ml/m² of body surface and EKG showed coronary insufficiency. Hypoxemia test was interrupted because of pain after five minutes and there were marked EKG changes. No exercise tolerance test was carried out. The B. M. R. was plus 16 per cent and the serum cholesterol 251 mg per cent. Methylthiouracil, 100 mg five times daily, was started August 1, 1946. After two and a half months' treatment he had improved. With decreasing doses of the drug this improvement continued and from the beginning of October, 1946 he had only occasional mild attacks of angina. A hypoxemia test, November 11, 1946, was unchanged and the exercise tolerance was 4,200 kgm. He started to work and felt fine. In the middle of April 1947 the hypoxemia and the exercise tolerance tests were unchanged. At the end of July 1947 he developed a slight myxedema with considerable enlargement of the thyroid gland. The drug was discontinued for one month and the attacks of pain returned gradually after the withdrawal of methylthiouracil. Treatment was resumed at the end of August, 1947 and he has since improved and is now, three months later, in fairly good condition and can do some work. The basal metabolic rate has varied between plus 29 and minus 9 per cent and the serum cholesterol, three months after institution of therapy has remained around 300 mg per cent.

Comment.

This patient with moderately severe angina belonged to Class III. He improved markedly after two and a half months' treatment and remained in good condition for another eight months. At that time the drug was discontinued for one month because of a slight myxedema and considerable thyroid enlargement. The symptoms gradually returned and since resumption of therapy he has improved again and is now in fairly good condition corresponding to Class II.

Case 9. R. E. G. (St. Erik's Hospital No. 4895/46), a forty-eight-year old female telephone operator, had had hypertension for three years when two years ago attacks of precordial pain and tightness with shortness of breath occurred. These attacks were brought on by slight exertion or emotional stress and she was unable to work. On admission August 1, 1946 the blood pressure was 190/110 and a faint systolic murmur and an accentuated aortic second sound was heard. Otherwise, physical examination was negative. X-ray of the chest showed an enlargement of the heart, the volume being 515 ml/m² of body surface and the EKG showed coronary insufficiency. Hypoxemia and exercise tolerance tests positive. The basal metabolic

rate was plus 18 per cent and the serum cholesterol 241 mg per cent. Methylthiouracil, 100 mg five times daily, was started September 2, 1946. After nine days' treatment she developed drug fever, and methylthiouracil was therefore replaced five days later by thiouracil. After two months' treatment she started to improve and after four months' treatment the improvement was considerable. With diminishing doses of thiouracil she has since remained in this condition for another ten months and still retained her work as a telephone operator five months ago. The basal metabolic rate has varied between plus 16 and plus 4 per cent, the serum cholesterol has been elevated being around 350 mg per cent.

Comment.

This case with moderately severe angina and hypertension belonged to Class III. She improved after two months' therapy and after four months' treatment the improvement was considerable and she has been able to retain her job. This improvement has lasted for another 9 months.

When methylthiouracil treatment was started five of our nine patients with angina pectoris belonged to Class IV, in two of these five cases a previous cervico-thoracic ganglionectomy had been done without lasting effect. The remaining four cases had a moderately severe angina belonging to Class III. In all except one patient, case 3, methylthiouracil medication proved therapeutically effective. After six weeks to five months' treatment a marked improvement of the subjective symptoms had occurred. This improvement then, with continuous treatment, on the whole remained unchanged in these eight patients; in cases 1 and 2, for instance, for more than twenty months. The improvement coincided in cases 1, 4, 5, 6 and 8 with a drop in the basal metabolism and in the subsequent course a continuous basal metabolic rate of about minus 15 per cent was aimed at. In the remaining three cases improvement occurred without the basal metabolism being lowered to any marked degree. Besides symptomatic response six cases also showed objective evidence of improvement during methylthiouracil treatment. The hypoxemia test was improved in all these six patients, in cases 4, 5, 7 it became negative. In four of these cases where repeated exercise tolerance tests were performed there was a considerable increase, by four to seven times in the amount of work these patients could tolerate.

Summary.

Of seven patients with congestive heart failure treated continuously over a long period with methylthiouracil, four were considerably improved and for a long time remained in this condition. Three patients showed moderate improvement. In two of these cases further therapy was stopped because of toxic reactions to the drug; the third case relapsed in spite of continuous treatment.

In eight of nine patients with angina pectoris there was a marked and lasting improvement during long standing methylthiouracil treatment. It was possible to record this improvement objectively during therapy. In most cases improved hypoxemia and exercise tolerance tests occurred.

As a rule, three to six months of treatment must elapse before improvement is noticed and with the dosage used, six to eight months of therapy were required before any reduction of the thyroid function could be clinically proved. When the thyroxin storage of the thyroid gland is exhausted it becomes easy to maintain the metabolic rate at a desired low level with very small daily doses (15 to 50 mg). We have aimed at metabolic values of about minus 15 per cent.

In most cases there was a coincidence of symptomatic response and of response of the basal metabolic rate. In five cases showing marked improvement, however, there was no particularly pronounced lowering of the basal metabolism. In these cases there was usually a rise in serum cholesterol indicating the blocking effect of the drug upon the synthesis of thyroxin. Thus a very low basal metabolic rate is not essential for benefit to result from the therapy in all cases. This finding is also in accordance with the view that thyroxin sensitizes the heart muscle to the anoxiating effect of epinephrine and that thiouracil exerts an opposite effect by suppressing thyroid hormone formation (4).

In cases of congestive heart failure and of angina pectoris refractory to other forms of therapy, treatment with thiouracil or allied compounds is of great value.

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A Comparative Study of Bile-Salts in Regard to their Influence on Mineral Metabolism.

By

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(Submitted for publication December 19, 1947.)

It is generally known that certain sterols play an important rôle in the regulation of mineral metabolism. Chief representative of this group is Vitamin »D», together with its precursors and derivatives. Another group of sterol derivatives also seem to have an influence on mineral metabolism, though their biological importance is known from another aspect. These are the bile-salts.

Data in the literature indicate that there is a rise in serum Ca, P, and Mg (1) following the injection of sodium cholate. It was shown that this substance diminishes the excretion of Ca in the feces (2) and increases the Ca excretion by the kidneys. Other experiments seem to prove that bile-salts facilitate the absorption of Ca from the intestine (3); however, studies, carried out by means of Ca isotope, brought out the evidence that Ca absorption from the intestine is very good (89.2 % of the Ca intake), and it therefore seems that the Ca concentration in the blood can not be taken as an indicator of Ca absorption (4).

It is the object of this paper to discuss whether the chemical structure of bile-salts plays a rôle in their influence on serum Ca, P, and Mg of the normal rabbit.

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Experimental.

The experiments were carried out in the winter of 1945, from October to February, throughout this period of testing the rabbits used were fed with a mixture of rye, soy-bean, and water. These circumstances must be mentioned, because presumably the food and the season itself may effect the reproducibility of the results. Rabbits of both sexes were used, of weights varying from 2.4 to 3.0 kg. The total serum Ca was determined from a sample of 0.2 ml serum, by means of the cerimetric titration, as worked out in collaboration with J. Richter (5). Inorganic phosphorus was determined from 0.5 ml serum, as described by Martland-Robinson (6), using a slight modification and magnesium as oxychinolate (7). The experimental procedure was as follows. Blood was taken from the marginal vein of the rabbit's ear. Then, 1 ml per kg body weight of the bile-salts solution in question (1 % of concentration) was injected subcutaneously. The rabbit's blood was tested again for Ca, P, and Mg, two hours after injection in one series, and three hours after injection in another series. 3 ml of blood proved sufficient for each of the combined analyses. The blood was centrifuged and used immediately afterwards for analysis. For the sake of control, it was established first whether or not the repeated removal of 3 ml of blood caused a change in serum Ca, P, and Mg. The changes in serum Ca, P, and Mg following bile-salt injection were then compared with these control experiments.

Results.

The results are summarized in the accompanying table. The numbers in the first column are those of the animals used. Each animal was used twice for experiments, and was always allowed to rest 3—4 days between experiments. The table is self-explanatory. The results are expressed in the absolute amounts of changes in serum Ca, P, and Mg in mg/100 ml serum. A comparison was made of sodium salts of cholic, taurocholic, dioxycholic, dehydrocholic and glycocholic acids. The table shows that cholic and taurocholic acids called forth a marked rise in serum Ca. The other effects are shown to be on a considerably smaller scale. An exception is the effect of Na-dehydrocholate

Table.

Animal Number	Ca (mg/100 ml)		P (mg/100 ml)		Mg (mg/100 ml)		Bile-salts inj. (0.01 g/kg body-weight)
	before inject.	change	before inject.	change	before inject.	change	
1	10.5	-0.1	6.26	0.20	4.52	0.17	Control: (No bile-salt) Changes in blood Ca, P, Mg, after 3 hours counted from the first blood-take.
2	10.4	0.0	6.11	-0.09	4.21	0.35	
3	9.13	-0.1	4.72	0.19	4.30	-0.30	
4	12.6	0.79	4.51	0.10	3.90	0.30	
5	12.5	-0.10	5.10	0.02	4.60	-0.09	
	11.7	0.10	4.70	0.30	3.80	0.31	
	11.6	0.20	6.61	0.11	4.10	-0.10	
	12.2	-0.10	6.00	0.11	3.20	-0.10	
	12.4	0.20	5.70	0.11	3.10	-0.10	
		-0.20	5.50	0.01	2.80	-0.10	
1	8.90	2.30	5.98	0.0	3.12	0.36	Na-cholate 2 hours after injection
2	8.80	2.30	5.62	0.09	3.00	0.85	
3	10.1	2.30	4.96	0.41	4.04	0.37	
4	7.90	2.30	4.80	0.11	3.60	0.40	
5	8.00	2.30	4.18	0.24	2.86	0.35	
			3.92	0.20	3.12	0.49	
	7.35	2.40	3.72	0.61	2.92	0.58	
	7.05	2.30	2.81	0.65	2.04	1.16	
	9.82	2.10	5.12	0.98	3.14	0.06	
	9.90	2.30	4.92	0.68	3.85	0.35	
1	11.18	2.70	4.05	0.13	2.26	0.18	Na-taurocholate 2 hours after injection
2	11.2	2.50	4.10	0.05	2.80	0.31	
3	8.81	2.10	4.35	0.24	2.17	0.27	
4	8.80	2.30	4.10	0.40	2.80	0.36	
5	12.0	2.90	5.38	0.18	2.79	0.61	
	12.2	2.60	5.10	0.29	3.00	0.50	
	10.80	0.0	4.28	0.38	2.47	0.03	
	10.2	0.10	4.00	0.25	2.52	-0.05	
	11.3	0.0	5.10	0.0	2.64	0.0	
	11.30	-0.10	5.10	0.0	2.52	0.09	
1	12.36	0.04	7.10	-0.10	4.60	0.60	Na-dioxycholanate 2 hours after injection
2	12.20	0.05	6.60	0.30	4.20	0.80	
3	13.30	-0.10	6.00	0.10	5.30	0.0	
4	13.20	-0.20	5.80	0.40	5.70	-0.60	
5	12.40	-0.40	7.10	-0.50	5.20	0.10	
	12.20	0.10	7.40	0.10	5.00	0.40	
	12.22	-0.12	3.90	0.20	5.10	-0.50	
	12.20	0.0	3.50	0.40	4.80	-0.30	
	9.82	0.02	4.30	0.10	4.10	0.30	
	9.79	0.02	4.20	-0.20	3.90	0.30	

Table. (Cont.)

Animal Number	Ca (mg/100 ml)		P (mg/100 ml)		Mg (mg/100 ml)		Bile-salts inj. (0.01 g/kg body-weight)
	before inject.	change	before inject.	change	before inject.	change	
1	13.70	0.05	7.33	0.52	3.42	3.03	Na-dehydrocholate
	13.76	-0.05	7.28	0.28	4.20	1.00	
2	13.40	-0.15	8.40	0.72	5.40	0.80	
	13.36	-0.06	7.82	0.08	5.00	1.95	
3	8.00	0.0	5.44	0.0	4.82	1.02	2 hours after injection
	7.88	0.22	5.00	0.11	4.90	0.60	
4	14.80	-0.14	6.54	0.06	5.80	0.30	3 hours after injection
	14.60	-0.20	5.89	-0.39	4.90	0.35	
5	13.46	-0.40	9.00	0.40	6.22	0.09	
	13.30	-0.30	9.80	0.20	6.45	1.15	
1	14.42	0.08	5.12	0.0	3.80	-0.20	Na-glycocholate
	14.38	0.06	5.10	0.10	3.20	0.70	
2	13.98	0.02	6.20	0.40	4.40	0.40	
	13.78	0.04	6.00	0.40	4.10	0.42	
3	11.18	0.03	6.30	0.25	3.80	0.30	2 hours after injection
	11.15	0.05	5.80	0.55	3.20	0.40	
4	10.50	0.10	6.12	0.0	3.30	0.40	3 hours after injection
	10.40	-0.10	5.00	0.86	3.50	0.50	
5	10.90	-0.20	6.50	0.50	4.30	0.10	
	10.70	0.10	5.90	0.50	4.00	0.60	

on serum Mg, two hours after injection, where it may be assumed that a considerable rise in the Mg concentration does occur, although this result is not consistently reproduced.

An analysis of these results brings out the interesting fact that Na-cholate caused, after two hours, an increase of 2.3 mg/100 ml of serum Ca in each case. This means that this effect is independent of the original Ca content of the serum. Three hours later this increase still remains unchanged. At the same time, serum P also shows a slight increase, which becomes more significant after three hours. Serum Mg seems to behave inversely, after two hours there is an increase followed by a decrease one hour later.

The effect of Na-taurocholate is similar to that of cholate, but the rise in Ca is somewhat higher, though not as uniformly regular throughout. Serum P and Mg show the same changes as in the case of cholate, but less marked. It is important to mention that the effect of taurocholate ceases after three hours. The other

bile-salts used here have no influence on serum Ca. Their effects on P show great individual differences, but a certain increase may be observed. Dioxycholanate and dehydrocholate also act on serum Mg, especially the latter, which causes a marked increase in Mg, as can be seen from the above. Glycocholate acts on non of these mineral constituents of the serum. Its slight tendency to cause an increase in serum Mg is neither uniform nor significant.

Discussion.

These observations seem to corroborate the data in the literature, and it can be stated that bile-salts have a well-defined influence on the mineral constituents of serum. However, this influence depends on the molecular structure of the bile-salts investigated here. Among these five bilesalts, only cholate and taurocholate had a significant effect on serum Ca. It must be pointed out that this increase in serum Ca does not depend on the original Ca concentration, and cholate causes in each case the same rise in serum Ca. The same is true in the case of taurocholate, but here the rise in Ca is somewhat higher and of shorter duration. This fact leads to interesting conclusions. It has been known that the serum Ca concentration depends on a great number of factors. It appears possible that certain sterol-derivatives, such as bile-salts, should play the rôle of some of these factors. It seems obvious that the increase of 2.3 mg of Ca per 100 ml serum must originate from bones or other tissues rich in Ca. If this is so, cholate and taurocholate must be regarded as factors responsible for the solubility of Ca in biological fluids. This assumption, while not as obvious, is nevertheless quite possible in the case of P and Mg. Other experiments now in progress support the preliminary supposition that derivatives of cholic acid influence the property of serum proteins to bind Ca ions, which fact seems to corroborate the above-mentioned theory. This problem calls for further detailed investigation.

The results of the foregoing experiments give only a general answer to the question raised at the beginning of this study. The trioxycholanic framework is seen to be represented in the bile-salts which are active on serum Ca. The combination of glycol and cholic acid inhibits this activity, while taurin (taurocholate) seems to intensify it.

Summary.

1. The effect of various bile-salts on serum Ca, P, and Mg of rabbits depends on their chemical structure.

2. The sodium salts of taurocholic and cholic acid are active on serum Ca, while P and Mg show a slight and more uncertain response to these substances.

3. The fact that 0.01 g/kg body weight of cholate and taurocholate raises serum Ca by a constant amount, which is independent of the initial Ca concentration, leads to the conclusion that these substances play a rôle in the solubility of Ca in serum.

I should like to express my thanks to the Factory Palik and Co. Ltd., to whose courtesy I am greatly indebted.

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Book Review.

Hanns Alexander: Differentialdiagnostische Bilder zur Lungentuberkulose. 146 pp. 127 illust. Georg Thieme, Leipzig 1948.

Hanns Alexander's works are clear in presentation and reliable. They are a pleasure to read and much profit is derived from them. In these respects this work does not differ from the preceding ones and therefore can be most warmly recommended. In a series of case histories with an abundance of radiograms of the lungs, an account is given of pulmonary lesions which are not infrequently confused with pulmonary tuberculosis. The differential diagnosis is carefully worked out for every case. The presentation might have been made still more fascinating if the real nature of the disease had not been disclosed before the case history was given, but only afterwards, as in that case the reader himself might have grappled with the problem for a while.

Erik Hedvall.



CARL SONNE,

Born April 18, 1882, died April 3, 1948.

Professor Carl Sonne was born in Allinge, Bornholm, but he was brought up in Svaneke. His parents were O. M. K. Sonne, merchant, and wife, née Kure. He was married to Marie, daughter of Consul Peter Petersen in Svaneke, born on December 10, 1882, who survives him. They had three children, Dr. Lass Mahler, Karen, married to Dr. Iver Aggerbeck, and Maja, married to Anker Kirkeby, Editor.

All his life Sonne felt closely attached to Bornholm. In 1941 he was elected chairman of the Bornholm National Society in Copenhagen and in 1944 of »Svaneke's Venner», another Bornholm society. After his last confinement to bed he went to his country home in Svaneke for convalescence and here he died suddenly of coronary thrombosis, after having got over an attack of the same disease a few months previously.

Sonne graduated in medicine in 1907. After serving his internship he worked several years in the State Serum Institute wherefrom he published his dissertation for the doctorate of medicine in 1914 — a now classical work on non-toxic dysentery bacilli. The Sonne dysentery bacillus is known to every medical student throughout the world.

In 1913—17 Sonne was resident physician to the Medical Department A of the Rigshospital, the University Clinic in Copenhagen, and in 1918—29 he was chief of the experimental laboratory of the Finsen Institute.

On the retirement of Professor Christian Gram in 1923 he competed with Drs. Chr. Lundsgaard, E. Hess Teisen and E. Meulengracht for the professorate in medicine thus vacant. Lundsgaard was appointed professor.

The following year Sonne was made chief of the Medical Out-patient Clinic of the Rigshospital, and when a new professorate of medicine was established in 1928, Sonne was appointed to this chair upon the recommendation of the University Faculty.

Sonne's interest in the lung function was aroused early, presumably through V. Rubow, a pupil of Christian Bohr, and as early as 1909, two years after his graduation, he published a methodological work on the registration of the respiratory phases. Already at that time he showed his ability to get along with very simple means, employing what has later been designated as servomechanics. On the whole, Sonne's technical faculties were considerable, and he greatly delighted in experimental work. Like the physiologists of old, he felt a keen desire to construct his apparatuses himself, and I have often seen him puzzling with a stick of sealing wax, some glass tubing, bits of rubber and pieces of a cigar box, building some very queer apparatus, which, after much patience, he finally got to work. Throughout his life he preserved this artisan's joy. This capacity for construction of apparatuses increased considerably with his increasing respiratory-physiological experiences. In 1932, in collaboration with

Einar Nielsen he worked out a particularly valuable technique for fractionated sampling of the alveolar air. Together with O. H. Christensen he constructed an apparatus for this purpose, which the latter employed to great advantage in his dissertation, which evidences a very considerable technical ability.

In 1915 and 1916 his work on respiratory-physiological problems led Sonne on to studies on the homogeneity of the air in the lungs — a question that is of decisive importance in estimating the method of Krogh and Lindhard for determination of the beat and minute volume of the heart. Here Sonne pointed out that the alveolar air is not such a homogeneous mixture as assumed by Krogh and Lindhard. This led on to a controversy in which Krogh and Lindhard were very outspoken. To me, Sonne appears to have been right in claiming that Krogh and Lindhard had overlooked certain sources of error in their method that assert themselves particularly in patients with pulmonary and cardiac lesions and which make determinations after Krogh and Lindhard's method uncertain for clinical estimation.

These investigations became of particular significance to Sonne's further work, however, and even up to his last days the problem of how the lungs are ventilated — the mixing of the air in the lungs, its inhomogeneity and the pulmonary circulation — was his pet theme, and he was particularly interested in the relation of this problem to emphysema of the lungs, asthmatic phenomena, silicosis and the various forms of dyspnea.

For elucidation of the air mixing in the lungs Sonne and his collaborators carried out some very laborious and exacting experiments, which have been published especially by Roelsen and by O. H. Christensen in their dissertations, involving chiefly the question of how inspired hydrogen was recovered in the expired air, and how oxygen and carbon dioxide were expired.

There can be no doubt that Sonne and collaborators succeeded in establishing that the lung does not function as a uniformly passive elastic group of alveoli, but these coworkers did not fully agree as to which parts of the lungs were ventilating the strongest. Nor is Sonne likely to have arrived at the final stand on this matter.

Even though in the literature it had already been suggested that the alveolar air was not to be looked upon as a homogeneous mixture, there is no doubt that it is Sonne and his school who have shown that this question involves some problem of great significance to the pathogenesis and clinical aspects of dyspnea.

Mention is to be made of one characteristic feature which encouraged Sonne to his studies on the unequal mixture of the air in the lungs. Sonne discovered early that the mixture of the air in his own lungs was particularly unequal. Gradually he realized that there was bound to be a constriction of one of his large bronchi and he associated this with an attack of pleurisy in his youth. On autopsy, performed by Dr. Gormsen, on his own request, Sonne was found to have been right in his diagnosis, for extensive adhesions were revealed in one pleural cavity, together with a lymph node that compressed a large bronchus. Thus the case history of Sonne confirmed once more that physicians may be able by self observations to make significant contributions to pathology.

Sonne and his pupils also endeavoured to overcome the difficulties connected with determination of the beat- and minute-volume of the heart, especially in cardiac patients, and the results of this work are presented in the dissertation of E. Nielsen (1937). These investigators succeeded in carrying through a technique which undoubtedly means a considerable advance beyond the acetylene method employed by Grollman. It is questionable, however, whether the recirculation through the coronary arteries might not in some degree jeopardize the method — as suggested by more recent experiences with catheterization of the heart and the direct method of Fick.

From Sonne's clinic, in 1939, T. Espersen published his dissertation comprising investigations on the volume of the circulation with employment of a method which largely corresponds to the one described by E. Nielsen and — as far as I can see — with this work he thus completed the programme set up by Sonne nearly 25 years previously.

Subsequently, P. P. Eskildsen (1945) published an interesting dissertation dealing with closely related problems.

Considering further that Sonne has published some excellent works on the width of the bronchioles, on cardiac dyspnea and on the arterial — and venous — CO_2 tension, there can be no doubt that Sonne has given some most valuable contributions to our present knowledge of the physiology and pathology of respiration and certain pathological aspects of the circulation.

During the decade in which Sonne was chief of the Laboratory of the Finsen Institute his remarkable gifts manifested themselves. It may be that the results of his studies on the metabolism of

flour-mites exposed to light are not quite incontestible, but in collaboration with Schultzer (dissertation, 1927) and Reeking he was one of the first to demonstrate the importance of light to the formation of vitamin D (calciferol) in the skin. Through a number of years this work was of the greatest significance to the treatment of rickets, and more recently it appeared to offer an explanation of the curative effect of the universal light-bath in lupus vulgaris — nay, possibly even the explanation of the effect of the local light-bath in this disease. Sonne brought up the question as to which parts of the light are biologically active; but even though this question gave rise to a valuable discussion it has not yet been settled. To Sonne it was a great disappointment that the studies he had commenced were not continued in the laboratory after he had left. Undoubtedly, however, some time it will be taken up again for further investigation.

Scientifically Sonne was very imaginative and also critical but not particularly productive in literary respects. He was somewhat «speculative», puzzling over his problems a good while. Several times he told me that he did not dare to read too much for when he started reading the problems would overwhelm him, and prevent him from concentrating on the tasks he had taken up. This critical imagination so readily aroused put its stamp on his teaching too. Again and again he would come back to the uncertainties of our knowledge and diagnoses. Least of all he was an examination coach driving a system into his students. He was far more like the Socratic midwife who wanted to lead the students on to recognition of the meaning of their own ideas.

Sonne's affection for his students was very strong, and to the very last his youthful mind found joy in their company. At the medical students' convulse their studies — the so-called Boserup celebrations — to which the university teachers are invited — Sonne just let himself go. He made speeches and he sang and he became a young student again — truly symbolizing that the universitas medicorum tam doctorem et discipulum mentioned in the statutes of the Montpellier University of 1220 is still alive in Copenhagen. Since 1938 he was chairman of the board of the «Studentergaarden» (a college), a trust he enjoyed and fulfilled admirably.

He always tried to limit his practice, but the patients he attended felt gratefully indebted to him.

In 1927—37 Sonne was a member of the Court of Invalidity Insurance and the last ten years of his life he was consulting physician to the insurance company »Denmark». He took a great interest in this work, and his broad clinical knowledge and critical sense enabled him to make a practicably valuable contribution to insurance medicine.

I have known Sonne from his first days as chief of the laboratory of the Finsen Institute, where I was his assistant and later I lived next door to him for half a generation. I can honestly say that I have never met a more amiable and obliging colleague. His geniality and friendliness were unsurpassed. His laughter was so hearty and joyous that once you heard it you could never forget it.

Sonne was no real orator and his style was often somewhat heavy and hesitant, sometimes even not altogether clear. This in connection with his gentle reticence and unselfishness has presumably brought about that he was hardly appreciated to the extent that his works entitled him to. But I am inclined to think that he is one of the Danish physicians who has contributed most

Contribution to the Knowledge of Arteriovenous Fistulas.

By

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(Submitted for publication January 14, 1948.)

Whilst physician in charge of the Medical Department II of the Sahlgrenska sjukhuset in Göteborg I had the opportunity to observe the following case, which for several reasons seems to be instructive.

I. S. H. woman aged 19. When aged about 13 the mandibula started protruding so that the incisives after a few years were to be found about $\frac{1}{2}$ cm anterior to those of the maxilla. She was operated upon for her prognathia Oct. 17th 1945 by Dr. G. Pettersson by means of the usual transection of the mandibular ramus. Dr. Pettersson, who is an expert of these operations, kindly told me that as a general rule no complications whatsoever are to be observed in connection with these operations, only are haematomas occasionally to be registered during the next few days. In this very case, however, no haemorrhage was to be noted during the operation and no haematoma afterwards. On the other hand, when the jaw was allowed to sink backwards, after transection of the mandibular ramus, there was noted a considerably impaired respiration, owing to an obstruction of the pharynx by means of the right tonsilla and necessitating emergency activities in order to get air. The teeth were wired as usual. Some few days after the operation the patient observed pulsations in the region of the right ear, synchronized with the beats of her heart. These throbbing and ponding sounds have increased during the last few months. She has had no pains whatsoever and besides of the mechanical and acoustic disturbances which have impaired sleep she has felt herself all right. No similar symptoms were to be observed previous to the operation.

The patient was seen by us in 1946, about 8 months after the operation. Her general condition was good, she had no cardiac compensation and the blood pressure was 145/90. X-ray examination of the heart disclosed essentially normal conditions, the size of the heart being reduced by the Valsalva test which also brought about a decreased rate. The electrocardiogram was normal, also when tested

by the Müller and Valsalva tests. In the right retromandibular region there was to be registered with the stethoscope, a typical continuous machinery-like murmur, a loud, whining noise with systolic intensification. The maximal intensity was below the tragus of the right ear but the murmur was to be listened in also in the temporal and the maxillar regions, always being synchronized with the pulse. If digital pressure was exerted upon the carotid artery on the right side of the neck the murmur immediately disappeared and the patient felt immensely relieved from the disturbing noise. When the throat was inspected there was a pronounced protrusion against the mid line of the right tonsilla. If a prolongation of the stethoscope was applied to this tonsilla the same loud machinery-like murmur was to be registered as below the right ear, disappearing as well if the carotid artery was compressed.

The diagnosis of an arteriovenous fistula was considered established. Whether or not the right half of the face was a trifle larger than the left might be discussed. The patient was sent back to Dr. G. Pettersson who tied off the external carotid on the right side, an intervention entailing immediate recovery from the symptoms. When seen by us about one month later the patient felt herself completely well, no murmur was to be registered and the protrusion of the right tonsil had disappeared.

In this case there seems to be no doubt about the presence of an arteriovenous fistula, reasonably established between the vascular province of the right external carotid artery and the corresponding veins, probably particularly between the system of the internal maxillary artery and the related venous outflow. The appearance of the symptoms in connection with the operation for prognathia makes it reasonable to consider the possibility of a traumatic etiology, as represented by the surgical intervention. There are, however, several reasons to doubt this explanation. Firstly, no haematoma whatsoever was to be observed in connection with the operation. Secondly, when haematomas occasionally have been noted in this connection, they have developed gradually and caused no impairment of the respiration as in this case where suffocation was imminent. Thirdly, the operation was performed by a surgeon extremely skilled and with a vast experience in this very line of surgery. It will hence seem reasonable to abandon the explanation of the syndrom as caused by the surgery and to assume a preformed vascular condition as responsible. It is well known that arteriovenous aneurysms may be encountered in the vascular province of the internal carotid, particularly

in the territory covered by the art. cerebri media. With regard to the external carotid our knowledge has so far been limited but it is perfectly obvious that a similar condition may be expected also when this artery is concerned, particularly perhaps in the region of the art. maxillaris interna, where the evolutionary conditions are complicated by the disappearance of art. stapedia and where a definite tendency to rete-formation is substantiated in those animals where this very part of the arterial system represents the source of the rete mirabile caroticum. Whether the establishment of such an arteriovenous aneurysm has anything to do with the prognathy seems uncertain; no similar observations have anyway been available from other cases of prognathy accordingly operated upon.

Arteriovenous fistulas as responsible for clinical symptoms and available to surgery have been known even since the reports of Halsted on the ligation of arteries. From practical medical point of view there are particularly six regions where such fistulas are to be expected:

1. Between the pulmonary artery and the aorta as ductus Botalli apertus.

2. In the head a) between the arteries and the veins of the brain, as in the so-called angiomas (*i. e.* arteriovenous aneurysms) of the convexity of the hemisphere, b) pertaining to the region of the external carotid, as exemplified by this case.

3. In the extremities usually as a result of traumatic injuries, such as gunshots.

4. It has been maintained that the circulation through the placenta is to be compared with an arteriovenous shunt causing added strain on the circulation not least if the delivery has been artificially accomplished (*f. ex.* by means of caesarean section).

5. It may be added that some instances of splenomegaly invite the conception of an arteriovenous fistula, although the hemodynamic mechanism only rarely may be so evident as in the cases some years ago reported by Hegler (portal malformation and intrahepatic arteriovenous aneurysm).

6. In Pagets osteitis deformans the vascularity of the bones is increased so as to make them act as free arteriovenous communications with resulting high cardiac output (Edholm et al.).

From patho-physiological point of view there are a few things to be remembered, which have been discussed in some detail al-

ready by Holman, of the Johns Hopkins School. Firstly, the heart is likely to be involved not only when the persisting ductus Botalli is concerned but also when other arteriovenous fistulas are about. The heart is likely to react with hypertrophy and dilatation for reasons developped by Holman and cardiac symptoms may cause the patient to apply for medical assistance. Secondly, there is always in such conditions a proximal dilatation of the artery, which is likely to increase as time passes on and which may be responsible for the enlargement of the extremity involved. Thirdly, the surgical obliteration of the fistula will always be followed by remarkable alterations of the general inculcation (reduced pulse rate, increased blood pressure), alterations subject, however, to gradual readjustments.

Whenever possible an abnormal arteriovenous communication should be closed by operation, the dangers otherwise being the cardiac complications, the local disturbances and the possibility of the shunt becoming the seat of a malignant infection with streptococcus viridans. In the present case a special danger may have been represented by the appearance of the right tonsill, which might have suggested the presence of an abscess and accordingly invited to incision, which obviously might have resulted in a fatality.

Summary.

1. A case is described, reasonably representing an arteriovenous aneurysm in the province of the right external carotid (internal maxillary artery).

2. A brief review is given of the arteriovenous fistulas as met with in the practical medicine.

3. Attention is called to the threefold danger of such fistulas, and to the necessity whenever possible to enter this »vibrant domain of surgery» as it was termed by Halsted.

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The Time of the Diazo Reaction in Laboratory and Clinic.

By

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Hijmans van den Bergh (14, 15) determined under certain conditions whether serum gives a visible discoloration within or only after 30 seconds of the addition of diazo reagent.

If a visible discoloration occurs within 30 seconds, Hijmans van den Bergh speaks of a direct reaction, otherwise he calls it an indirect reaction.

Besides a difference in the rate of the reaction with diazoreagent Hijmans van den Bergh found other differences between direct and indirect reacting bilirubin, such as a difference in solubility in chloroform, a difference in the rate at which bilirubin is oxidized and a difference in the degree of adsorption on the albumin precipitate which occurs when albumin is withdrawn from serum.

Hijmans van den Bergh found indirect reacting bilirubin in the sera from normal people and from patients with an icterus caused by an increased breakdown of the blood, while sera from patients with a so-called obstructive jaundice proved to contain direct reacting bilirubin. Later on it appeared that patients with an impaired liver may also have direct reacting bilirubin in their sera without any existing biliary obstruction.

The explanation which Eppinger (8) gave for it was not satisfactory. The bile thrombi which he described were not always found. Furthermore it was seen that an important part of the biliary ducts may be blocked without any icterus resulting from it (Rich), so that, if the bile thrombi should be the cause of the icterus in patients with an impaired liver, these bile thrombi should also be found in a great many bile capillaries.

This was mostly not confirmed by the facts.

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In the serum of the liverless dogs of Mann and Bollman (20, 21, 22) indirect reacting bilirubin was found. These dogs got an icterus in which the bilirubin, formed outside the liver, could not be excreted by the liver. So there was an obstructive jaundice and direct reacting bilirubin was expected in the blood.

These experiences induced Rich (24) to propose another classification of the icterus. Instead of contrasting the obstructive or the mechanical icterus, with the haemolytic or the dynamic jaundice, Rich took the passing or not passing of the liver cell as a standard.

Bilirubin that has passed the liver cell, reacts directly. This bilirubin may come into the blood if the barrier between biliary ducts and blood system is broken.

This is the case with obstruction in the biliary ducts in consequence of a mechanical obstruction of the bile-discharge, but also in consequence of a necrosis of the liver cell when this cell has been severely impaired.

In these cases Rich spoke of a regurgitation jaundice.

Bilirubin that has not passed the liver cell, reacts indirectly. An excess of this bilirubin may be found in patients with an increased breakdown of the blood, but also in those cases where the liver cell fails in its function of excretory organ of bilirubin.

This icterus was called retention icterus. Thus we find a retention icterus in patients with a pernicious anaemia, a congenital, haemolytic icterus, but also in patients with a so-called familial non haemolytic jaundice, with a deranged excretion of bilirubin caused by the liver cell, which was proved with the bilirubin tolerance-test (3, 12).

In young patients with an erythroblastosis foetalis we find a combination of factors as is proved by the bilirubin tolerance-test and the increased haemolysis.

Also in patients with a pernicious anaemia and a haemolytic icterus, the liver cell will fail in its function as excretory organ of bilirubin, especially if the anaemia has grown worse, owing to which fact, besides the increased haemolysis, a diminished excretion of bilirubin may also be of importance in these diseases. This factor always plays a part in patients with heart decompensation and pneumonia. An-oxaemia of the liver cell causes a diminished secretion of bilirubin (23). The serum bilirubin reacts indirectly in patients who are in this stage of the icterus.

Only if the impairment of the liver increases so much that the liver cell dies, the diazoreaction becomes direct. This may sometimes even occur in patients with a pernicious anaemia.

With an obstructive jaundice in consequence of a mechanical obstruction of the bile discharge, indirect reacting bilirubin in the serum is often found at the onset of the disease. In that stage of the icterus the obstruction of the biliary ducts is not such that bilirubin gets from the biliary ducts into the blood-vessels. It is true that in that case the liver cell fails in its excretory function, so that too little bilirubin is excreted.

We shall see later on that in case of biliary obstruction the diazo reaction in the serum soon becomes too rapid, without the reaction being »direct» already, if the method of Hijmans van den Bergh is applied.

The Diazo Reaction.

This reaction was carried out by Hijmans van den Bergh in the following way: $\frac{1}{4}$ — $\frac{1}{2}$ volume of diazo reagent is added to one volume of serum plus one volume of water. The diazo reagent consists of a mixture of 10 ml of »diazo A» (sulphanilic acid 12 ml, hydrochloric acid 25 %, 15 ml, distilled water to bring the volume up to 1,000) and 0.3 ml of »diazo B» ($\frac{1}{2}$ % sodium nitrate solution).

This mixture should be freshly made. If a red discoloration is visible within 30 seconds, the reaction is direct, otherwise it is indirect. Soon after Hijmans van den Bergh's publications it appeared that some sera still give a clear red discoloration in just over 30 seconds after the addition of diazo reagent, while other sera show a discoloration which is less visible after a much longer time. The first case was called a diphasic reaction (9, 18b), the second case an indirect reaction.

This reaction is said to be found especially in sera with mixtures of direct and indirect reacting bilirubin (31). This conception was accepted for a long time without any criticism.

Why the direct reacting bilirubin, which is present in the serum, gives a discoloration after only 30 seconds, is not clear.

It will be proved that most probably there are not two kinds of bilirubin, *but that bilirubin may react with diazo reagent at entirely different rates*. It depends on the quantity of bilirubin in the serum *and* on the rate of the reaction, whether a visible discoloration is seen within or after 30 seconds.

Watson (24) studied the curves which are obtained, if the rate of the diazo reaction is determined with the Malloy & Evelyn method.

These curves are often very vertical in the beginning but become horizontal quite suddenly. Watson now thinks that »the change from an almost vertical to a much more horizontal line represents a change from one order of reaction to another, thus indicating the presence of two substances».

My objection to this reasoning is that we do not obtain two straight intersecting lines if we draw the curves on logarithmic paper.

It is still possible however, that mixtures will occur, consisting of a component which reacts rapidly and another which reacts slowly. The one reacting rapidly will react at various rates in the various sera. This interpretation of the curves is supported by the experiments made by Bungenberg de Jong.

Bungenberg de Jong proved that *the colloidal condition in which bilirubin appears in the serum, determines the rate of reaction of the bilirubin* (1). The present writer has enlarged these experiments (6).

As there are gradual transitions from one colloidal condition into the other, it is easy to understand that bilirubin may react in quite different ways. These different ways of reacting do not only concern the rate of the reaction with diazo reagent, but also the solubility in chloroform, the degree of oxidizability, of adsorption on an albumin precipitate.

Consequently the investigator, studying the degree of solubility in chloroform, did not determine the quantity of indirect reacting bilirubin, but the condition of the bilirubin in the serum. So we cannot separate direct from indirect bilirubin or determine the quantity of one of these substances, but we can only distinguish some qualities of the bilirubin appearing in the serum, such as the rate of the reaction with diazo reagent (6).

The Determination of the Rate of the Diazo Reaction.

The rate of the diazo reaction is influenced by a great many substances. Many substances accelerate the rate of the reaction. Especially the rate of the reaction with indirect reacting bilirubin increases by the addition of these substances.

This accelerating action is made use of to determine the bilirubin percentage in a serum. By adding a sufficient quantity of such a substance we may achieve that all the bilirubin present in the serum, is diazotized within a reasonable time, say 15 minutes.

Thus Hijmans van den Bergh availed himself of the accelerating action of alcohol. Later on a far better substance was used viz. caffeine.

As the reaction with indirect bilirubin especially, increases by adding such substances, the difference in the rate of the reaction of direct and indirect reacting bilirubin diminishes in consequence of the addition of substances which accelerate the diazo reaction. Too little allowance is mostly made for this, and so it was possible for some investigators to assume as recently as 1943 that bilirubin always reacts approximately at the same rate with diazo reagent and that the direct or indirect reaction is only dependent on the degree of the icterus (10).

The influence of the pH and of the salts on the rate of the diazo reaction was studied by Bungenberg de Jong (1). He discovered that:

1. By changing the pH of a *delayed reacting serum* to about 5, an increase in the rate of the reaction may be observed. At higher as well as lower pH values a decrease in the rate of the reaction takes place. *Direct reacting serum* has its maximum rate of reaction at pH 4.5.

2. In the presence of buffers the bilirubin reaction of delayed sera becomes more or less direct in the pH area 7—5.5. The influence of a buffer on the rate of the reaction may be reduced to two distinct factors.

- a. a change of the pH of the medium.

- b. a specific effect of every ion on the solvation of the serum colloids.

All this makes it clear why Gray and Whidborne (9), found such slight differences in the rate of the reaction between direct and indirect reacting bilirubin. Later on these investigators omitted urea in determining the rate of the reaction. They continued to use a buffer, however. Also the determination of the bilirubin percentage with the Rappaport & Eichhorn method gives rise to faulty conclusions, because this method, especially in sera with indirect bilirubin, gives values which are far too low, also if we wait 24 hours instead of 15 minutes before reading the extinctions. Thus I found the following figures in a few sera, while comparing this method with the one of Jendrassik and Grof (17).

	Jendrassik & Grof	Rappaport & Eichhorn
Icterus catarrhalis	3.7 mg	3.05 mg
Weil's disease	3.4 »	3.1 »
Erythroblastosis foetalis	3.85 »	1.55 »
Erythroblastosis foetalis	20.2 »	3.9 »

Another factor which is not often taken sufficiently into account, is the slower reaction of direct bilirubin, if serum is diluted with water. If we want to find the greatest differences in the rate of the reaction in various sera, no substances should be added which accelerate the diazo reaction and we should dilute with as little water as possible. The rate of this reaction may be determined according to two different principles:

First of all we can stop the reaction in various tubes at certain moments by adding a substance which retards the diazo reaction.

With (26) thought that he could stop the reaction by adding a strong alkaline liquid. While this liquid is added the reaction becomes so rapid for a moment during the rapid rise of the pH, that a not insignificant quantity of bilirubin is converted during the addition of the liquid. The following example will elucidate this:

2 ml of distilled water and 0.5 ml of diazo reagent are added to 1 ml of serum. After 1 minute the extinction of the red azobilirubin solution is determined in the Stufen photometer by means of filter S. 53. From the extinction-concentration curve we can read how much bilirubin was diazotized in one minute.

To another sample of the above-mentioned mixture 1.5 ml of alkaline solution (10 g of NaOH + 35 g of K-Na-tartrate + 100 ml of distilled water) was added one minute after the addition of the diazoreagent. The extinction of this mixture was determined by means of filter S. 61.

Now it proved that for instance of a serum containing 1.6 mg of bilirubin, approximately 0.15 mg was diazotized in one minute if no alkaline solution was added, and 0.95 mg if this was added, while of a serum containing 4.6 mg of bilirubin, 0.7 mg and 1.5 mg respectively were diazotized in one minute. That is the reason why the method of With is incorrect.

In the second place the extinctions of a mixture of serum and diazo reagent may be measured from moment to moment *e.g.* every 15 seconds. From this we can calculate how much bilirubin was diazotized every time.

Malloy-Evelyn (19) and Jendrassik-Cleghorn (16) set about it in this way. Especially Malloy-Evelyn, however, dilute excessively with water and the result of this is, as was already explained before, that the differences in the rate of the reaction between the various sera are not so manifest. Another drawback of diluting to such a high degree is, that the extinctions which are to be measured in sera with only little bilirubin, become very slight, so that

these extinctions are not very well measurable, especially in consequence of the faint opalescence of the mixtures. Therefore I did not dilute with water and set about it in this way (Room temperature: 20° C.).

In cuvette A one volume of serum and one volume of diazo reagent were brought together (10 ml of a mixture of 1 gram of sulph-anilic acid + 15 ml of 25 % hydrochloric acid to 1 litre of water = diazo A and 0.3 ml of $\frac{1}{2}$ % sodium nitrite solution = diazo B).

In cuvette B, I brought together one volume of serum and one volume of diazo A.

After exactly 15, 30, 45 and 60 seconds and after 2, 3, 4, 5 and 10 minutes or longer the extinction of the azobilirubin solution was read. The extinction-concentration curve was made according to the method of Hijmans van den Bergh. In this way it is possible to calculate how much bilirubin in the above mixture of serum and diazo reagent was diazotized in a certain time.

If the bilirubin concentration of the same serum is determined, we can calculate how many per cent of the bilirubin is diazotized in a certain time.

In sera with a high bilirubin percentage, the diazo reaction of which is very rapid, we cannot determine this rate at all or only for a very short time, because after that time the extinctions of the azobilirubin solutions become greater than 2.

The following examples clearly show the greater difference in the rate of the reaction in various sera with the method described above and with the Malloy & Evelyn method.

Bilirubin percentage	1 minute percentage according to the Malloy & Evelyn method	1 minute percentage according to my own method
4.6 mg.....	15 %	15 %
3.1 »	24 %	39 %
15 »	26 %	43 %
4 »	37 %	66 %

The measuring-instrument that was used, was the Stufen photometer of Zeiss.

The Determination of the Bilirubin Percentage of the Serum.

Generally only those methods are used now, which do not cause any formation of an albumin precipitate.

Malloy & Evelyn add methyl alcohol; Jendrassik and Grof

add caffeine and a buffer as substances which accelerate the reaction.

With found far higher values with the method of Jendrassik and Grof than with the Malloy & Evelyn method (75).

Ducci and Watson (7) think that this results from the addition by With of alkali to the azobilirubin solution.

The objections raised by Ducci and Watson are essentially right.

While comparing the methods of Jendrassik & Grof and those of Malloy & Evelyn, the following figures were found:

Patient suffering from	Bilirubin percentage according to Jendrassik & Grof	Bilirubin percentage according to Malloy & Evelyn
Icterus catarrhalis	2.65 mg	2.5 mg
Erythroblastosis	6.65 »	5.6 »
Pernicious anaemia	2.65 »	2 »
Congenital non-haemo- lytic hyperbilirubin- aemia	6.25 »	5.1 »
Liver cirrhosis	4 »	3.3 »
»	0.95 »	0.95 »
»	4.25 »	3.6 »
Hepatitis	30.5 »	26 »
Icterus catarrhalis	7 »	7 »
» »	8.25 »	7 »

From this we can see that the Jendrassik & Grof's method gives somewhat higher values than the Malloy & Evelyn's method.

This difference may be a consequence of the fact that serum-bilirubin is better diazotized by a caffeine-buffer than by alcohol. There are good reasons to consider this very likely, as With found that a certain bilirubin solution is diazotized in a much shorter time by caffeine and that the extinctions which are obtained, are higher in case of caffeine.

With's observation that after serum has been added to a bilirubin solution we obtain higher extinctions after diazotization than without any serum, points to the fact that we do not ascertain the real concentration in the serum when we determine the bilirubin percentage.

Neither would this be the case if we make an extinction-concentration curve with bilirubin added to serum, as »serum bilirubin» is different from »bilirubin which is added to serum».

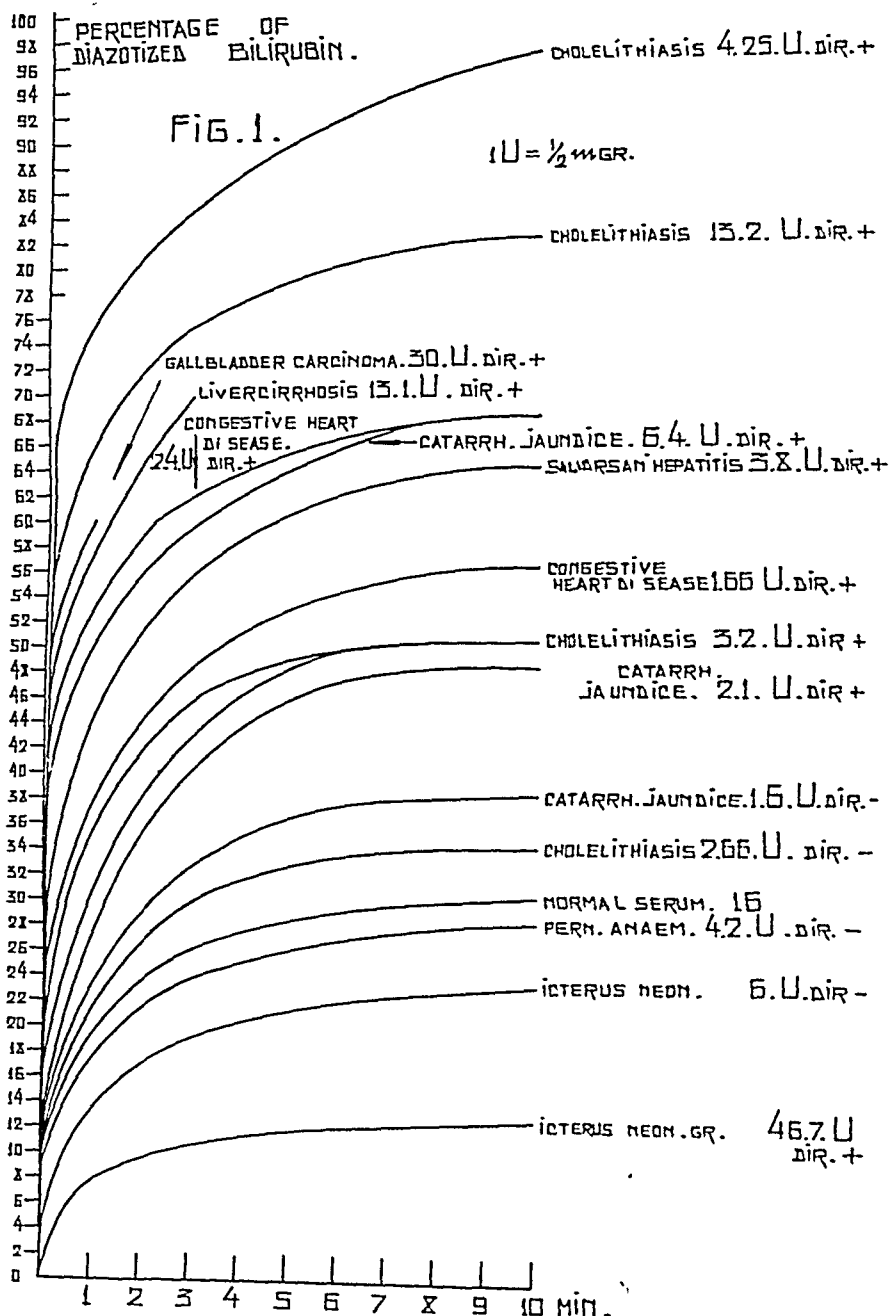


Fig. 1. Determination of the rate of the diazo reaction in various sera.

Results and Discussion.

The rate of the diazo reaction of a large number of sera was determined at 20° C. Fig. 1 renders some curves which were ob-

tained by plotting out the percentages of diazotized bilirubin on the vertical axis and the time during which the diazo reaction took place on the horizontal axis.

The curves of fig. 1 do not intersect. So we can say that the percentage of bilirubin which is diazotized, say in one minute, is a standard for the rate of the reaction.

The determination of the »one minute percentage» and not of the say »ten minute percentage» is prompted by practical considerations.

The extinctions of sera with much bilirubin which reacts rapidly, are, as a rule, no longer determinable after one minute. I did not venture upon a more thorough analysis of the obtained curves, because

a. The diazo reagent is not excessively present in sera with more than 25 mg of bilirubin.

b. While studying the sera of patients with delayed reacting bilirubin (pernicious anaemia, icterus neonatorum and icterus neonatorum gravis) we generally found that the reaction was more delayed as the bilirubinaemia was stronger. Fig. 2 renders this very clearly.

c. Great modifications of the pH change the condition of a colloidal solution. This change takes time. Diazo reagent is a strong acid solution which causes a considerable fall of the pH.

Bungenberg de Jong (1) proved that the colloidal condition determines the rate of the diazo reaction. This colloidal condition may change under the influence of the fall of the pH after the addition of the diazo reagent. Nothing is known of the time in which the colloidal condition changes. Neither do we know anything about the influence of those factors on the course of the diazo reaction.

A further study of this problem will have to decide whether the above influence does exist and how great that influence is on the course of the diazo reaction. For the time being one thing and another urge us to be very careful in analysing the obtained curves.

The curves of fig. 1 show us that except in the above-mentioned sera with indirect bilirubin, the rate of the diazo reaction is not connected with the bilirubin percentage of the serum.

If, in case of the qualitative reaction of Hijmans van den Bergh, we find that bilirubin reacts directly, it does not imply that the bilirubin in that serum is also rapidly diazotised. If the bilirubin

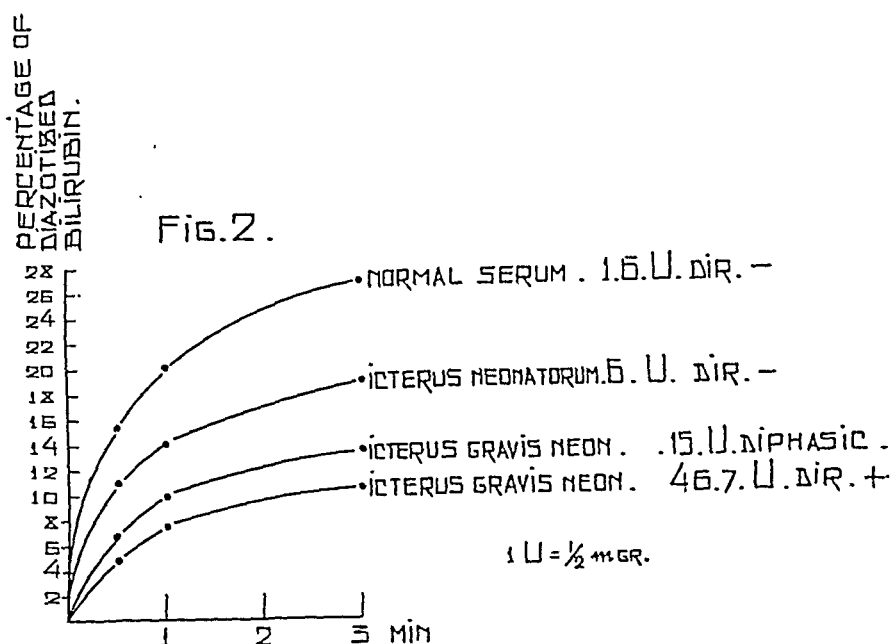


Fig. 2. Determination of the rate of the diazo reaction in sera from patients with an increased breakdown of the blood.

percentage of the serum is high we find a direct reaction, while the bilirubin may still react very slowly indeed.

Particular attention should be paid in this connection to curves, rendering the examination of sera from babies with an icterus neonatorum and an icterus neonatorum gravis. The curves 2 a and b render the examination of the serum from a baby, dying three days after its birth in consequence of an icterus neonatorum gravis.

Curve 2 a renders the examination of the blood from the umbilical cord. The diazo reaction proved to be diphasic. The curve shows that the rate of the reaction is not greater than in sera from normal people or patients with a haemolytic icterus.

Curve 2 b renders the examination of the serum which was obtained by a heart puncture immediately after the death. The diazo reaction proved to be direct. The curve of this serum too runs horizontally.

These observations explain some different statements about the syndrome of the icterus neonatorum gravis.

Some investigators always find an indirect reaction, others often find a direct reaction. This direct reaction was only apparently direct, however.

Waugh, Merchant and Maughan (26) found the following results with the aid of the Malloy & Evelyn method:

In sera from babies with an icterus neonatorum, 0.06—0.94 mg % of bilirubin was converted into azobilirubin within 10 minutes. In sera from children without an icterus neonatorum they found approximately the same values. In an icterus neonatorum gravis much higher values were found, however, for the bilirubin percentage and for the quantity of bilirubin which was diazotized in 10 minutes. These investigators thought that the occurrence of these larger quantities of bilirubin which reacts within 10 minutes, could only be explained by assuming that thrombi are formed in the biliary ducts of these babies. This is an example how one may arrive at incorrect conclusions, if one, as is generally done at the moment, determines *»how much»* bilirubin is diazotized in a certain time and not *»how many per cent»* of bilirubin is diazotized in a certain time.

If the investigators mentioned above had determined the percentage they would have found in their patients suffering from an icterus neonatorum gravis, that the diazo reaction in these patients is rather slower than quicker compared to the diazo reaction in normal babies. The error mentioned above occurs quite often as people still stick to the notions of direct and indirect bilirubin, by which they understand two kinds of bilirubin which are distinctly separated.

We have seen that bilirubin may react *»directly»* if it reacts very rapidly but also if it reacts very slowly.

Fig. 3, rendering the examination of sera with diphasic reacting bilirubin, shows, that the rate of the diazo reaction is also quite different in these sera.

What advantages does the determination of the rate of the diazo reaction offer, as it was described above, over the Hijmans van den Bergh's method?

Our insight in the diazo reaction is much deepened by it. I have availed myself repeatedly of this method in studying the cause of the different reactions of bilirubin in serum (3, 4). The study of the diazo reaction during the course of an icterus is not very well possible without this method (5).

In our clinics we shall be able to avail ourselves successfully of this method in those cases where the icterus is slight and the diazo reaction *»indirect»*, while yet an obstructive jaundice or an impairment of the liver is thought possible. Quite often we shall find too rapid a reaction in those cases.

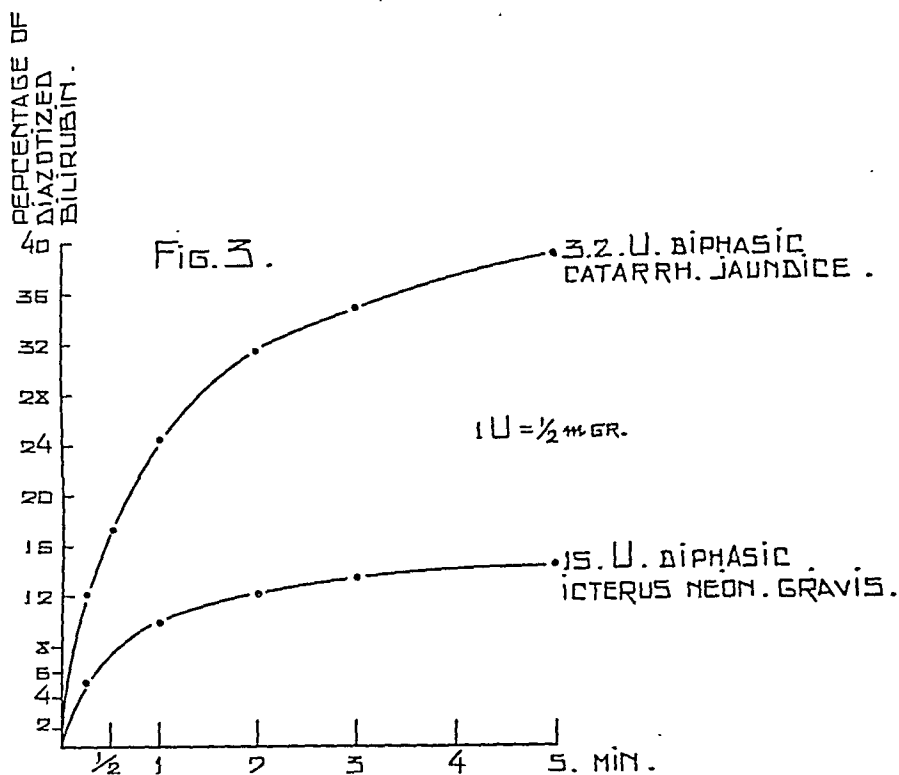


Fig. 3. Determination of the rate of the diazo reaction in sera with diphasic reacting bilirubin.

It proved that a one minute percentage of more than 20 % in sera with a bilirubin percentage of more than 1 mg (25 % in sera with a bilirubin content between $\frac{3}{4}$ and 1 mg) is never found in patients with a haemolytic or pure retention icterus (in the meaning of Rich).

So a one minute percentage of more than 20 % means too rapid a reaction (provided the method described above is used). Sera with less than $\frac{1}{2}$ mg of bilirubin are not very well suited for further study.

There have been investigators who tried to examine sera with a bilirubin percentage of less than $\frac{1}{2}$ mg with an electric colorimeter. Others, however, doubt whether the low extinctions which have to be determined here, are easily measured, if the Malloy and Evelyn's method is used. The present writer will return to these problems in a special article.

Summary.

Hijmans van den Bergh found that after adding diazo reagent to serum, he sometimes obtained a visible discoloration within 30

seconds and sometimes not. He distinguished between direct and indirect reacting bilirubin.

Apart from different reactions with diazo reagent, Hijmans van den Bergh found other differences: solubility in chloroform, oxidizability and adsorption on the albumin precipitate caused by treating serum with alcohol.

Afterwards it was thought that many sera contained mixtures of direct and indirect bilirubin.

Many investigators have tried to determine the relative quantities of direct and indirect bilirubin in a given serum from the solubility in chloroform or from the quantity of bilirubin giving the diazo reaction in a given time.

Indirect bilirubin is not completely soluble and there is a difference of opinion about the time required for the diazo reaction of direct bilirubin.

The more accurate determination of the rate of the diazo reaction rather than the judging from the appearance or non-appearance of a change in colour, suggests that probably there are not only two kinds of bilirubin, but that bilirubin may react in many different ways with diazo reagent.

It is possible that mixtures will occur, consisting of a component which reacts rapidly and another which reacts slowly. The one reacting rapidly will react at various rates in the various sera.

This tallies with the experiments by Bungenberg de Jong and by Deenstra, who showed, that the rate of the diazo reaction is determined by the colloidal condition of the serum; as it is known that gradual transitions between several colloidal conditions exist.

In determining the rate of the diazo reaction, inaccurate methods were often used which led to faulty conclusions.

Some years ago many investigators still believed that there were no differences in the rate of the diazo reaction. The time of the diazo reaction should be determined in a watery medium, while care should be taken that the presence of substances which accelerate the reaction is avoided as these substances especially influence the direct bilirubin.

Another important factor is the degree of dilution with water which affects the rate of the diazo reaction.

A method of estimating the time of the diazo reaction in a mixture of equal quantities of serum and diazo reagent is described.

The percentage of bilirubin diazotized in one minute at 20°C is used as the method of expressing the rate of the reaction.

The determination of the quantity of direct bilirubin is useless as there are no standards for deciding what direct bilirubin exactly means. We can only determine how bilirubin reacts in a certain serum.

The quantity of direct bilirubin will vary according to the time factor and degree of dilution with water. In sera with a bilirubin content of more than about 1 mg%, a one minute percentage of more than 20 % means too rapid a diazo reaction if the present writer's method is used. Too rapid a reaction is found in patients with a regurgitation jaundice.

The estimation of the one minute percentage makes Hijmans van den Bergh's experiences more useful clinically.

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Sex Hormones and Leukemia.¹

By

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(Submitted for publication December 17, 1947.)

It has been demonstrated by numerous experimental investigations that a certain relationship exists between the sex hormones and leukemia. This relationship is far from having been fully elucidated, and the problem on the whole is complicated by the fact that divergent results are obtained by experiments carried out in the same manner, if different, inbred strains of mice are used. This must be interpreted to the effect that the result of the experiments depends on the genetic constitution of the mice which in the highly inbred strains must be considered fairly constant, but varying from strain to strain.

The presence of a sex factor is apparent *int. al.* from the higher female than male incidence of leukemia in several strains. In the case of mice the type of leukemia is rarely stated in detail; undoubtedly it is usually a question of lymphosarcoma, *i. e.* a lymphogenous leukemia of a rather acute type. Considering that mouse leukemia is dominated to a great extent by generalized or localized lymphosarcoma, it is improbable that the slight variation from strain to strain of the sex ratio in leukemic mice should be explicable by the potential, but small variation in the occurrence of the different types of leukemia. A factor of more decisive importance in judging the sex ratio in leukemic mice is the deviating predominance in each individual strain of all females (healthy and leukemic) and the ordinarily briefer lifetime of the females. In this matter too, however, much depends on the

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strain. Crossing C 58 with Sto-Li (Storrs-Little) MacDowell, Potter & Taylor (1945) found that on an average the females survived the males, whereas Furth in 1946 states that the mortality curves for other strains show that most males survive their leukemic sisters.

In the strain (?) used by Mercier (1937) leukemia occurred in 38.2 per cent. of the males, but in 60.3 per cent. of the females. Cole & Furth (1941) deal with the problem in more detail and arrive at the conclusion that the predominance of leukemia in females is unquestionable, although rarely significant. In one strain (F₁ Ak/Rf) the males were more frequently affected with leukemia than the females. Without going into details they state that on an average the females become affected with leukemia at an earlier age than the males and furthermore, that the incidence of leukemia among females remains uninfluenced by pregnancy (percentage of leukemia for bred mice 72.8, for virgins 70.5). Lefèvre (1945), on the other hand, working with strain AKa, found a higher incidence of leukemia in bred (61 per cent.) than in virgin females (54 per cent.). The difference is not, however, significant. The difference between the incidence in females (60 per cent.) and males (52 per cent.) is not either significant. Thus, the predominance of leukemia in females is rarely marked, but it appears to be unquestionable, because it is rather constant. This observation is not surprising, as it is known that estrogens may be extremely leukemogenic.

Lacassagne, in 1937, was the first to induce (accelerate) lymphosarcoma with an estrogenic substance on 14 mice from various strains. Since then his observation has been confirmed by int. al. Shimkin, Grady & Andervont (1941) in strain C, Bischoff, Long, Rupp & Clark (1942) in strain March-Buffalo. Gardner, Kirschbaum & Strong, in 1940 demonstrated that the estrogenic effect in strain C₃H was nullified by androgens. Gardner, Dougherty & Williams (1944), using various estrogens, were capable of confirming Lacassagne's experiments in the case of strains CBA, C₃H, PM, A, JK, C₁₂₁, but in one strain (C₅₇) estrogen had no leukemogenic effect. The incidence of leukemia was found to be highest following administration of large doses of hormones, but the effect of estrogen was nullified by testosterone propionate.

McEndy, Boon & Furth (1944), working with strain AK, reduced the incidence of leukemia from 74 per cent. to 45 per cent. by ovariectomy (on mice aged 23—56 days), but following

orchidectomy (on mice aged 20—56 days) the incidence of leukemia rose from 52 to 60 per cent. Murphy (1944) operated with a strain (Rockefeller Institute Strain — R. I. S.) with an incidence of leukemia of 88.4 per cent. in females and 53.5 per cent. in males. Ovariectomy did not reduce the incidence, but mice treated with testosterone and submitted to ovariectomy exhibited an incidence as low as 58.4 per cent. Orchidectomy, on the other hand, increased the incidence from 53.5 per cent. to 97 per cent., a fact indicating that androgen inhibits the development of leukemia and therefore acts as an antagonist to estrogen. Similarly, following orchidectomy before puberty and following a later treatment with estrogen, Dmochowski & Horning (1947) found 59 per cent. and 70 per cent. leukemic males respectively in 2 mixed stocks. The controls, non-castrated, but also treated with estrogen, developed leukemia in only 11 and 19 per cent. of the cases respectively.

It is not known with certainty whether the estrogenic substances have a direct effect on the hemopoietic tissue. Their inhibitory effect on the pituitary as well as the relationship with the adrenals are well known. In addition, estrone is of course responsible for the development of the secondary sex characters and has a marked influence on the growth of the female genitals, that is an influence on the growth of a large number of different tissues some of which influence the hemopoiesis.

(1) Writer's Investigations of Mice.

A study was made of the incidence of and mortality from spontaneous leukemia in 2,766 mice of the inbred strain AKa. The mice were left untreated and observed until they died spontaneously. As far as possible their conditions of life were identical.

Of the mice 69.7 per cent. were females; the sex ratio was thus 2.31 in favour of the females. Leukemia was observed at the age from 4 to 22 months. Those dying before the age of 4 months have therefore been left out of the material. Of 1,931 females 1,134 (= 58.7 per cent.) died from leukemia at an age ranging from 4 to 20 months. Of 835 males 458 (= 54.8 per cent.) died from leukemia at an age ranging from 6 to 22 months. Accordingly, the incidence of leukemia is slightly higher among the females than males (the difference is not, however, significant, as $\chi^2 = 3.58$ and $0.10 > P > 0.05$) despite the fact that non-leukemic males live slightly longer than non-leukemic females, and this is another

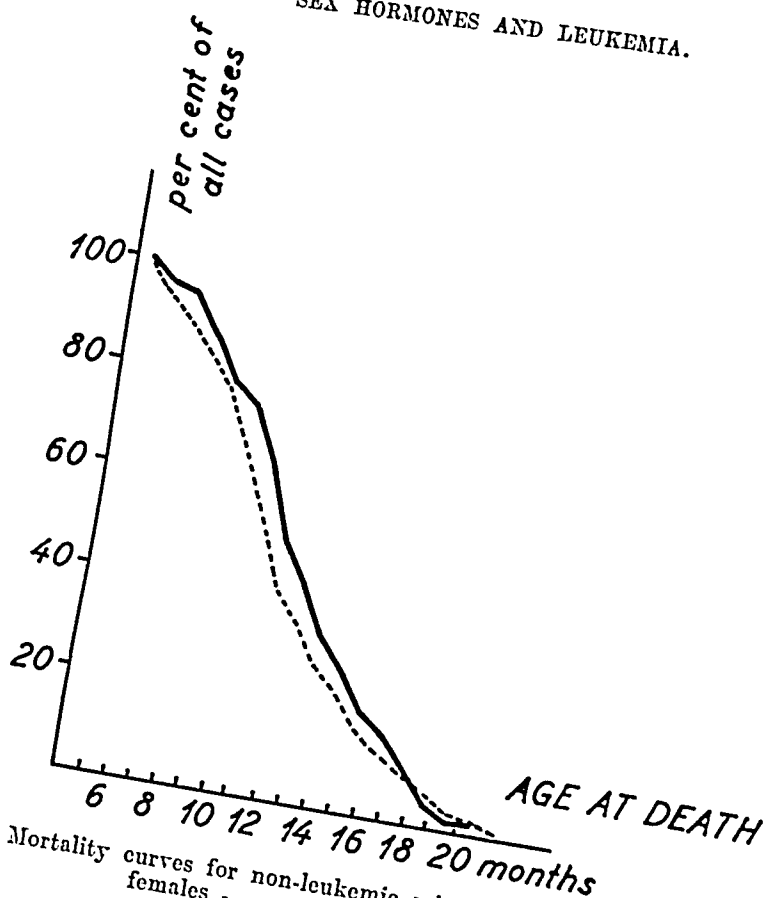


Fig. 1. Mortality curves for non-leukemic mice (377 males — and 797 females - - - -) of strain AKa.

reason why the males are more exposed to leukemia. Of the 1,134 females 630 had bred or were pregnant, whereas 504 never had bred. The incidence of leukemia was the same for bred and for virgin mice (59.1 per cent. and 58.2 per cent. respectively).

Hyperplasia of the thymus gland was observed in 130 = 6.7 per cent. of the females (average age at death 9.4 months) and in 36 = 4.3 per cent. of the males (average age at death 10.1 months). Therefore, the females die earlier and more often from hyperplasia of the thymus than the males.

It is apparent from Fig. 2 that on an average the females die earlier from leukemia than the males. The difference is highly significant, as $\chi^2 = 32.20$ and $0.01 > P > 0.001$. It is, however also evident from the same figure that mice that have bred or are pregnant do not die of leukemia earlier than the virgin mice ($0.20 > P > 0.10$), when the females dying after having reached the age of 15 months are ruled out.

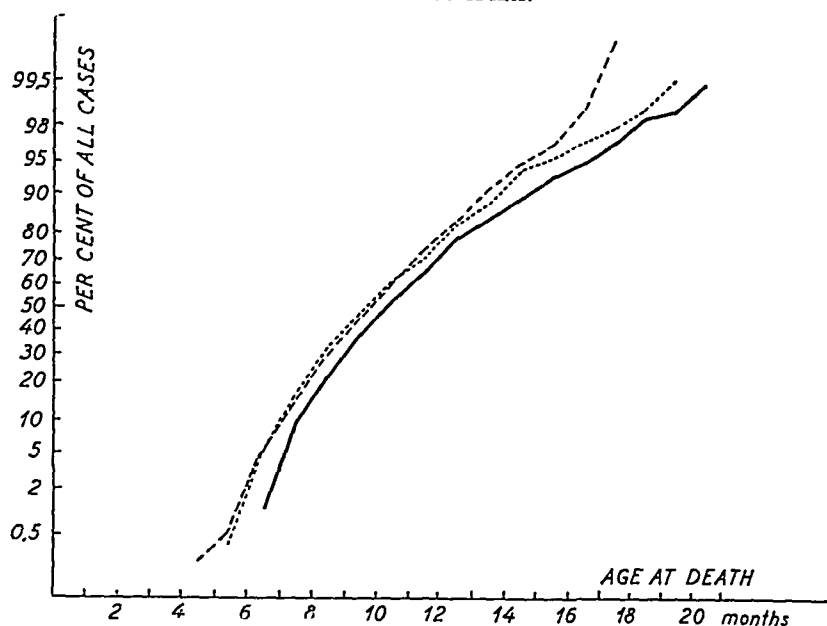


Fig. 2. Probits diagrams showing the age at death of leukemic mice of strain AKa (468 males ———, 504 virgin females and 630 females that had been through one or more pregnancies).

From this may be concluded that there is in females of strain AKa — regardless of pregnancies — one or several factors partly causing an earlier manifestation of leukemia, partly a higher incidence of leukemia. In other words: In females there are factors accelerating leukemia or else there are in the males factors delaying the development of leukemia. Correspondingly, hyperplasia of the thymus gland is found more often and at an earlier age in the females.

On the basis of the experimental results published hitherto the most probable explanation is an estrogenic acceleration of leukemia and hyperplasia of the thymus gland in females, although a slight inhibition of the development of leukemia presumably plays a part in the male organism on account of its output of androgen.

(2) Writers Investigations of Man.

In human leukemia too the sex factor plays a part. It is evident from the sex ratio applying to the various types of leukemia. In the case of acute leukemia in children the sex ratio appears to

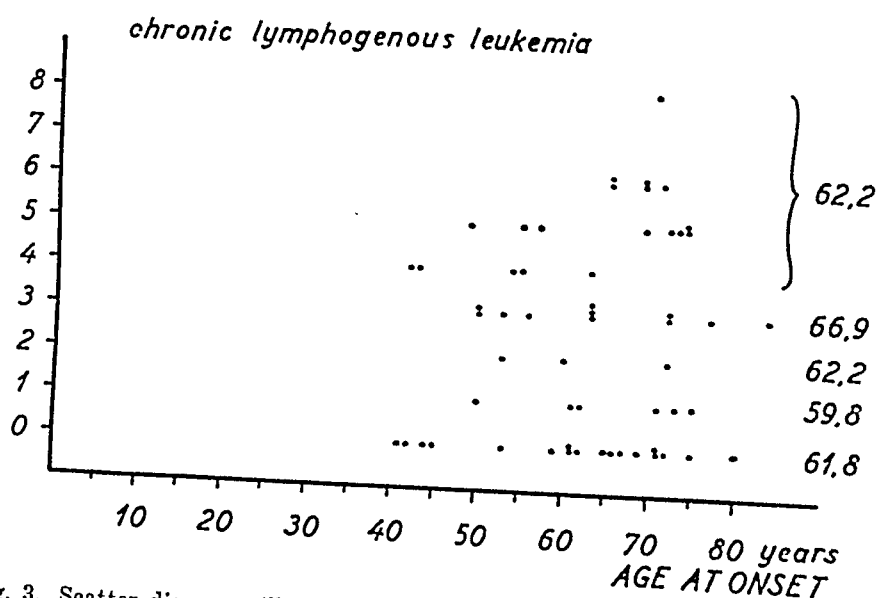
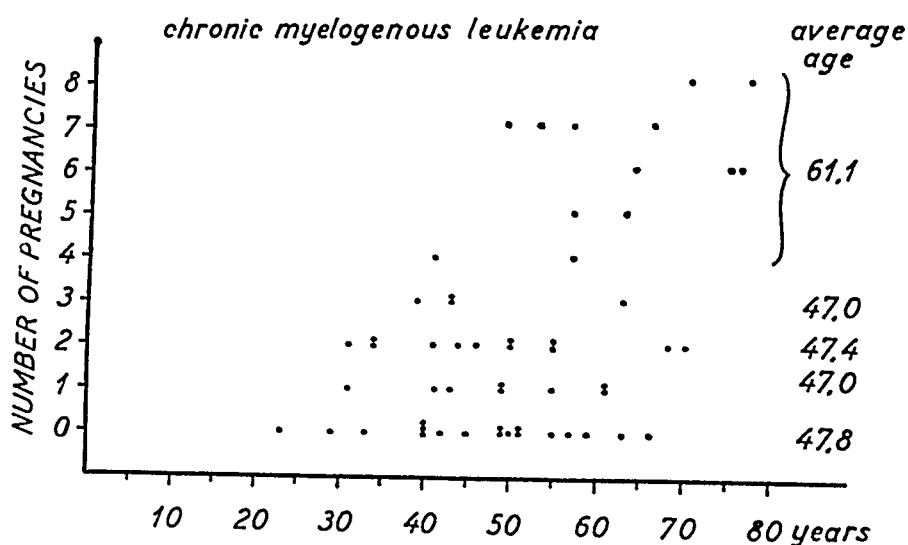


Fig. 3. Scatter diagrams illustrating the age distribution in cases of chronic myelogenous and chronic lymphogenous leukemia in women never pregnant and in women who have been pregnant one, two, three or more times.

depend on the age of the individual: during the first year of life acute leukemia is more common in girls, but later the disease prevails in boys, the more the older they are (Cooke 1942). Chronic lymphogenous leukemia is about twice as common in men, whereas

chronic myelogenous leukemia is slightly more common in women (int. al. Minot, Buckman & Isaacs, 1924).

A study (Videbæk, 1947) on the basis of 310 unselected cases of human leukemia gave a corresponding result, revealing the following sex ratios (men/women): for chronic lymphogenous leukemia $74/53 = 1.4$, for chronic myelogenous leukemia $33/46 = 0.7$, and for acute leukemia $53/49 = 1.1$. Furthermore, the disease proved to occur at almost the same age in men and women. The scatter diagram (Fig. 3) illustrates the age upon manifestation of chronic lymphogenous and chronic myelogenous leukemia in women who have never been pregnant and in women who have been pregnant one, two, three or more times. Accordingly, women who have been through one or more pregnancies do not acquire leukemia earlier than women who have never been pregnant. On the contrary, the latter appear to have a tendency to a somewhat earlier onset of leukemia. The difference is not, however, significant.

The reason why man does not exhibit the same conditions as mice of inbred strains is probably that in man it is a question of other types of leukemia. In addition, experimental investigations emphasize the importance of the genetic constitution which is fairly constant in the same strain of mice, whereas it is extremely varied from one human being to another.

Summary.

(1) It appears from the literature that inbred strains of mice usually exhibit a higher incidence of leukemia in females than males and that estrogens accelerate leukemia, whereas androgens inhibit its development.

2) In strain AKa the incidence of leukemia is highest in females which also acquire leukemia at an earlier age than males. Pregnancies do not accelerate leukemia.

(3) In man leukemia occurs at almost the same age in men and women. Pregnancies do not appear to hasten the development of leukemia.

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On the Influence of Immune Hemolysin on Red Blood Corpuscles in Vivo and Vitro.

By

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(Submitted for publication December 17, 1947.)

Earlier Investigations.

The influence of hemolysins in vivo was a question of current interest already in the beginning of this century. In 1906 Donath and Landsteiner (9) described, in cases of paroxysmal hemoglobinuria, a kind of hemolysin which became activated in the cold. In 1908—1909 Chauffard and co-workers (4 and 5) stated iso-hemolysin in serum in two cases of non-hereditary hemolytic jaundice. This hemolysin acted at room temperature and bodily temperature and they considered this the cause of the intravital hemolysis. It was inactivated on heating to 56° C, and reactivated if complement was added. v. Stejskal described autohemolysis in the blood of patients with acquired hemolytic jaundice. Hemolysins have since repeatedly been demonstrated in this disease, but not regularly. In some cases autoagglutination (8, 21) has instead been observed. Similar reports have also been made in cases of Lederer's anemia (12). In respect of the etiology of the pathologically increased hemolysis the same significance may be attached to autoagglutination as to autohemolysis. In both cases the essential lies in that substances which affect the red blood corpuscles in vivo have been stated. It is, some way, perhaps a case of studying the phenomenon from two different angles. In hemo-

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lytic states, caused by the rhesus factor, only the agglutination phenomenon can as a rule be stated *in vitro*.

These problems of extremely great interest attracted many investigators early in the present century. Widal and his collaborators should principally be mentioned (22). After the First Great War these questions were forgotten, however, but during latter years a lively interest has again been evoked in these problems, particularly among hematologists in America. Foremost among these are Dameshek and Schwartz who in 1940 (8) published an excellent survey of acute hemolytic anemia, a conception comprising both acquired hemolytic jaundice and Lederer's anemia which are considered as one and the same disease. They are of the opinion, that the disease is caused by hemolysins in the blood and question whether the etiology of familiar hemolytic jaundice is not similar, although hemolysin is not generally stated in the latter disease.

A few years earlier the same authors (7) succeeded in bringing about an acute hemolytic anemia in animals which seemed to be identical with that of man. They used immune hemolysin which they obtained by immunization of rabbits with blood corpuscles of guinea pigs. The immune serum obtained was injected intraperitoneally or intramuscularly into guinea pigs. Depending on the size and number of doses the animals sickened in acute or subacute hemolytic anemia. Similarly as in hemolytic anemia in man the red blood corpuscles changed shape, the diameter being decreased and the thickness increased. The resistance to hypotonic salt solutions decreased simultaneously. They also stated that the hemolysins acted more strongly *in vivo* than *in vitro* but they did not investigate the question in detail. The animals that died of anemia had an enlarged spleen.

Similar tests were made already in 1913 by Banti (1). He used dogs and rabbits for his tests. He, too, found that immune hemolysin acts more strongly *in vivo* than *in vitro*. Banti furthermore compared the intravital influence on normal and splenectomized animals. In the latter case the effect of the hemolysins was much weaker. Also toluylendiamine acted less strongly after splenectomy but aqua destillata acted equally strongly independently of splenectomy having been performed or not. After injection of immune hemolysin or toluylendiamine an initial, rapid hemolysis was observed and a slow hemolysis, setting in later. Particularly the latter was weaker following splenectomy.

The hemolytic influence of acetylphenylhydrazine is, on the other hand, according to Singer (19) and Cruz and others (6), equally effective after splenectomy as before it.

Ponder and co-workers (17) examined, in 1941, the effect of saponin and some other hemolytically acting substances *in vivo* and *in vitro* and arrived at the remarkable result that the effect of these hemolysins is two hundred times stronger *in vitro*. They only studied, however, the direct effect on the red blood corpuscles and did not take an interest in their later fate in the organism. According to these authors the stronger effect *in vitro* is due to 1) A higher cell concentration *in vivo* than in common titration methods *in vitro*. 2) The active substance does not react *in vivo* only with the erythrocytes, it is also absorbed by some of the other cells. 3) The plasma proteins inhibit the influence of the hemolysin. A continuous new-formation of hemolysin *in vivo* may, on the other hand, be influential in the opposite direction.

The Problem.

We know that when the red blood corpuscles are influenced by a hemolytically acting agent they first change into a more spherical shape (8). On the other hand we also know that during their passage through the spleen the erythrocytes change in the same manner (3, 10, 13), and that the spherocytosis in patients with acquired hemolytic jaundice disappears after splenectomy and decreases at least, in persons with congenital hemolytic jaundice (8, 14). On that account we cannot say with certainty whether the spherocytosis in hemolytic anemia with demonstrable hemolysin in the blood is a direct sequence of the effect of hemolysin on the blood corpuscles. The second possibility is that the hemolysin only affects the blood corpuscles in a manner which facilitates the organism (principally the spleen?) to change their shape into a more spherical one, and later to dissolve them. This may also be said of the spherocytosis in experimental hemolytic anemia, as in Dameshek's and Schwartz's tests with animals. For the purpose of illustrating this question I have compared the change of shape of the blood corpuscles after injections of hemolysin into normal animals, into splenectomized animals, and *in vitro*.

As already mentioned, Banti (1) stated that a certain amount of hemolysin destructs a decidedly greater number of red blood corpuscles in normal than in splenectomized animals. He further-

more observed that the amount of dissolved blood corpuscles in the test-animals is much greater than would be expected according to the titer of the hemolysin in vitro. The same observation was made also by Dameshek and Schwartz. They used repeated intramuscular or intraperitoneal injections, while Banti gave each test-animal one intravenous injection. However, these authors compared the blood cell count in vivo with a titration result in vitro — a fact which perhaps robs the result of some of its value. On that account I have endeavoured to compare the effect of a certain amount of hemolysin in vivo and in vitro in, as far as possible, similar conditions. The same measuring scale, *i. e.* blood corpuscle count, was used in each case.

Technique.

Adult guinea-pigs were used for the tests. Some of them were splenectomized. The surgical operation certainly was simple from a technical point of view and yet the majority of the animals died a few days or weeks after the intervention, and the post mortem generally gave no indication as to the cause of death. On that account the material was less extensive than desired. Seven guinea-pigs survived the intervention for a period long enough to allow them to be used as test-animals. Different anesthetics were used, for instance, ether, urethane, avertin and ether, and urethane and ether. The last-mentioned method seemed to be the most suitable. About 0.3 ml of a 25 per cent urethane solution per 100 grams of body weight was administered intraperitoneally, and later ether was given very cautiously. The abdomen was opened with median cut. The stomach was drawn out and shifted to the right, the spleen, which in the guinea-pig lies close to the ventricle, following along. When the vessels of the spleen had been ligated with catgut the organ was removed and the abdominal wound closed with catgut. After the intervention the animal was immediately placed in a thermostat at 37° C for a few hours.

Immune hemolysin was produced by immunizing a rabbit with washed guinea-pig blood corpuscles, as described in detail by Dameshek and Schwartz (7). The hemolysin was also titrated according to these authors. A 2 per cent suspension of guinea-pig blood corpuscles was used. The final serum dilution in the last test tube, in which hemolysin was distinctly visible, was given as a titer. The immune serum obtained had the hemolysin titer 1 : 386 at 37° C. It remained unchanged in repeated check-ups during the period the tests were made. The agglutinin titer was stronger, *viz.* about 1 : 1500 at 37° C. The serum was generally used in a dilution of 1 : 4 which thus had a hemolysin titer of about 1 : 100.

This kind of hemolysin was given intraperitoneally to some test-animals in repeated doses, and to others a dose was given once only, direct into the blood. There is some trouble in making a venous puncture in guinea-pigs and it requires anesthesia which, again, may effect the reaction of the animals. Instead of intravenous injection, intracardial injection was employed. It is easily done and does not require anesthesia. After the injection control of the point of the needle still being in the correct position in the heart was made by aspiration.

Blood Tests.

All blood samples were taken through a small slit above an ear vein. The red blood corpuscles were enumerated according to common praxis in Bürker's counting chamber. The volume percentage was determined according to van Allens hematocrite method. The erythrocyte diameter was measured on dry films with ocular micrometer. The mean corpuscular volume was calculated according to the formula

$$\frac{\text{Volume per cent}}{100 \times \text{R. B. C. per cmm}} \times 10^9 \text{ c}\mu$$

and the mean thickness according to the formula

$$\frac{\text{mean corpuscular volume}}{\pi \times \left(\frac{\text{mean diameter}}{2} \right)^2}$$

The reticulocytes were stained with brilliant cresyl blue.

The following normal values from 11 animals were obtained:
Red blood corpuscles 4—6 millions/cmm.

Vol. per cent 35—45. Mean corpuscular diameter 7.2—7.7 μ , mean volume 80—90 $\text{c}\mu$, and mean thickness 1.7—2.0 μ . Reticulocytes 3—5 per mille.

The *blood volume* was determined according to the »Evan's blue» method (11, 16), somewhat modified for use of Pulfrich's photometer, and adapted for guinea-pigs. The dye solution was used in a concentration of 1 : 20,000. Pulfrich's photometer equipped with an absorption filter No. 61 and a micro-cup of a depth of 20 mm was used for reading the dye concentration in serum. Instead of venous puncture heart puncture was used in this case similarly as in hemolysin injections. Mild anesthesia was given to keep the animal quiet. Blood in a quantity of 2 ml was taken and 2 ml of the dye injected. The same quantity of blood was again taken 3—5 minutes later. Both blood samples were centrifugated, the serum separated, and the extinction coefficient of the stained serum sample determined. The second cup contained unstained serum. A calibration curve which had earlier been prepared for dilutions corresponding to 10—60 ml serum was used for reading the serum amount. The hematocrit value had been de-

terminated beforehand, and the blood serum amount was calculated according to the formula $\frac{100}{100 - \text{volume per cent}} \times \text{serum volume}$.

Repeated Intraperitoneal Hemolysin Injections.

Repeated intraperitoneal injections of hemolysin containing serum of the titer 1 : 100 were given to 4 guinea-pigs. In a few days three of them acquired hemolytic anemia of the same type as Dameshek's and Schwartz's test-animals. In addition to a decrease of the erythrocyte count, leucocytosis and change in shape of the red blood corpuscles was stated. Their mean diameter decreased and their thickness increased, while the volume remained unchanged. A day or two later large polychromatic erythrocytes, basophilically punctured corpuscles, and normoblasts appeared in the blood. Vital staining revealed at this stage a considerable reticulocytosis, and the diameter measurement an extensive dispersion with abundance of microcytes and macrocytes. When the injections were terminated the microcytes decreased in number, and the mean diameter increased rapidly.

Two test-animals of the same size, the one with the spleen intact, the other splenectomized earlier, were each given 0.2, 0.2, 0.3, 0.4, 0.5 ml daily, and after an interval of two days an additional 0.3 and 0.3 ml per day. The red blood count of the former was reduced from 6.07 to 3.07 millions, while that of the latter decreased only from 5.5 to 4.47 millions. The splenectomized animal's blood corpuscles showed no distinct change of shape. The mean diameter of the second animal's erythrocytes was, however, reduced from 7.13 to 6.45 μ and the number of microcytes (blood corpuscles with a diameter of 5 μ or less) was increased from 0.5 to 24 per cent (See Table 1).

Into two other animals, of which again the one lacked the spleen, 0.3 ml of serum was injected intraperitoneally once daily during six days. Also in this test the erythrocyte count and the shape of the corpuscles changed much less in the splenectomized animal. The blood corpuscles of the former were reduced from 4.82 to 1.53 millions, those of the latter from 4.66 to 3.03 millions per cmm. The mean diameter of the erythrocytes decreased 0.94 μ in the animal whose spleen was intact, and in the splenectomized animal 0.47 μ , while the thickness increased as much as 1.4 μ in the former animal, but only 0.4 μ in the latter (See Table 1 and Diagr. 1).

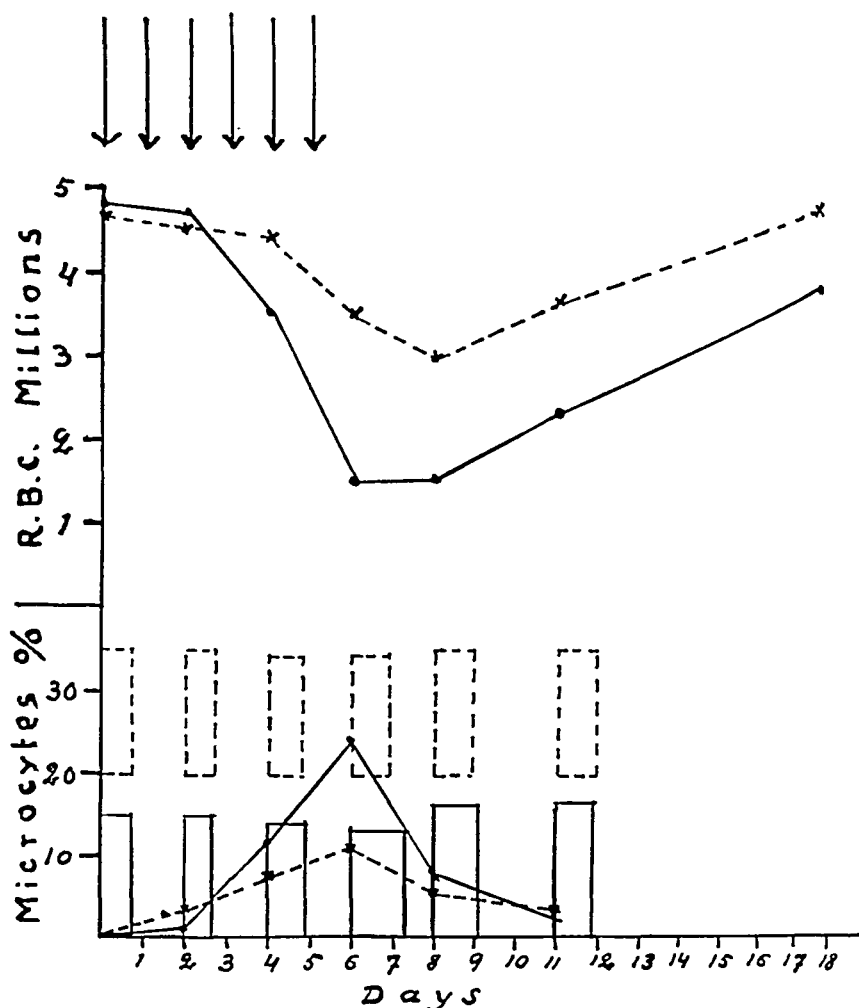


Diagram 1. A normal (uninterrupted line) and a splenectomized (dotted line) guinea-pig each received, during six days, daily intraperitoneal (arrows) doses of 0.3 cmm of hemolysin containing serum of the titer 1:100. The upper curves denote the number of red blood corpuscles per ml, the lower ones the percentage of microcytes (blood corpuscles with the diameter $5\ \mu$ or less). The rectangles denote the mean dimensions of the blood corpuscles, the height denoting diameter and the breadth the thickness.

Intracardial Injection of Hemolysin.

Fifteen guinea-pigs were given single intracardial doses of hemolysin. The smallest dose was 0.08 ml serum of the titer 1:50

Table 1.

The effect of repeated intraperitoneal hemolysin injections on the red blood corpuscles. Initial = initial value. Min. = lowest value measured. Max. = highest value measured. Figures in brackets denote time elapsed after institution of treatment.

No.	Weight	Dosage	R. B. C. per cmm		Microcytes		Mean diam.		Mean thickness		Reticulocytes	
			Initial	Min.	Initial	Max.	Initial	Min.	Initial	Max.	Initial	Max.
1 (spleen intact)	600 g	0.2-0.5 ml \times 7	6.07 mill.	3.07 mill. (7 days)	0.5 %	24 % (5 days)	7.13 μ	6.45 μ (5 days)	?	2.14 μ	7 $\%$	207 $\%$ (5 days)
2 (spleen-ectomized)	625 g	"	5.05 mill.	4.47 mill. (4 days)	2.5 %	2.5 %	7.23 μ	7.23 μ	2.0 μ	2.0 μ	1 $\%$	56 $\%$ (9 days)
3 (spleen intact)	385 g	0.3 ml \times 6	4.82 mill.	1.53 mill. (6 days)	0 %	24 % (6 days)	7.46 μ	6.52 μ (6 days)	1.9 μ	3.3 μ (6 days)	17 $\%$	115 $\%$ (6 days)
4 (spleen-ectomized)	330 g	"	4.66 mill.	3.03 mill. (8 days)	0.5 %	11 % (6 days)	7.50 μ	7.03 μ (6 days)	1.9 μ	2.3 μ (6 days)	14 $\%$	99 $\%$ (6 days)

per 100 g body weight. In this case no reduction of red blood cell count was obtained. All the other fourteen test-animals were given hemolysin of the titer 1 : 100 in doses varying between 0.05 and 0.2 ml per 100 gm body weight. All these animals acquired a typical hemolytic anemia. The changes in the blood were not, however, proportionate to the doses, they differed greatly in the various test-animals. For instance, the animal obtaining the dose 0.05 ml per 100 g acquired an anemia with 1.35 million Eryth./cmm in three days and died in five days. The animal which was given 0.075 ml per 100 g had only a moderate, transient anemia with a red blood cell count of 3.55 millions in four days. Three guineapigs receiving doses of 0.125, 0.14, and 0.15 ml, respectively, per 100 g died already in about 24 hours of extremely severe hemolytic anemia accompanied by hemoglobinemia and hemoglobinuria. Those who died all had a much enlarged spleen. For instance, that of the animal that died of anemia following a dose of 0.05 ml per 100 g weighed 2.9 g after death while the weight of the normal spleens removed varied between 0.3 and 0.9 g.

Blood samples were taken in general 1, 3, and 6 hours following the injection, in twenty-four hours and, later, once daily. Hemolysis was not immediately observed — not until an hour or so later. As seen from Table 2, a moderate decrease of the red blood cell count was observed in one hour in about 50 per cent of the animals, in three hours in the majority of cases (77 per cent), and in six hours in all of them. Already before the blood corpuscle count was reduced a diminution of the mean diameter and an increase of the thickness was as a rule observed (See table 2 and Diagr. 2).

Table 2.

Hemolysis during the first six hours following an intracardial hemolysin injection. The mean values include only those cases in which the respective changes were observed.

Time elapsed after injection	Total number of cases	Decrease of R. B. C.		Decrease of mean corpuscular diameter		Increase of mean corpuscular thickness	
		Number of cases	Mean decrease	Number of cases	Mean decrease	Number of cases	Mean increase
1 hour..	8	4 (50 %)	0.35 mill.	7 (88 %)	0.39 μ	6 (75 %)	0.2 μ
3 hours.	13	10 (77 %)	0.44 mill.	12 (92 %)	0.66 μ	12 (92 %)	0.4 μ
6 hours.	7	7 (100 %)	0.51 mill.	7 (100 %)	0.84 μ	7 (100 %)	0.5 μ

If the animals survived the anemia was in general at its severest in three—four days. Simultaneously, or somewhat earlier, the spherocytosis was at its peak. The thickness of the red blood corpuscles increased greatly as a rule, in a few cases to the double. The volume of the blood corpuscles did not change in general. In four to five days a considerable reticulocytosis was observed. It generally reached the values of 10—30 per cent. As the reticulocytes are remarkably large cells, both the mean corpuscular diameter and the mean corpuscular volume increased parallel to the reticulocytosis. In initial reticulocytosis there was still an abundance of microcytes and on that account there was great dispersion of the diameter measure. The microcyte count was later reduced, and the mean diameter increased, generally to values above the initial value.

Five of the fourteen animals which were given intracardial hemolysin injections of the titer 1 : 100 had earlier been splenectomized. In parallel tests (See Table 3) these animals always reacted less strongly than did the normal animals receiving a similar dose. As already mentioned, the influence of hemolysin varied greatly in the various cases. Also the difference between the reaction of the normal and the splenectomized animals differed greatly. Following a dose of 0.1 ml per 100 g both acquired moderate anemia, the difference between the two being quite insignificant. The maximum decrease of red blood corpuscles in the splenectomized animal was 1.53 millions and in the normal one 1.71 millions per cmm, in each case after a period of five days. The difference was somewhat greater regarding the spherocytosis. All animals with the spleen intact and receiving larger doses than 0.1 per 100 g died, but among the splenectomized animals only the one died which was given the largest dose used, viz. 0.2 ml per 100 g. The difference was greatest in the doses 0.125 and 0.15 ml per 100 g (See Diagram 2). The splenectomized animals certainly both acquired a severe anemia which culminated in six and three days respectively, but the non-operated animals died in slightly more than one day showing severe hemoglobinuria and hemoglobinemia. The blood changes following the doses 0.14 ml per 100 g were almost parallel, but whereas the splenectomized animal recovered, the second one died in seven days. After the dose 0.2 ml per 100 g both animals died two and a half days later, yet the hemolysis was decidedly less severe in the splenectomized animal. This animal not surviving for a longer period is due to the

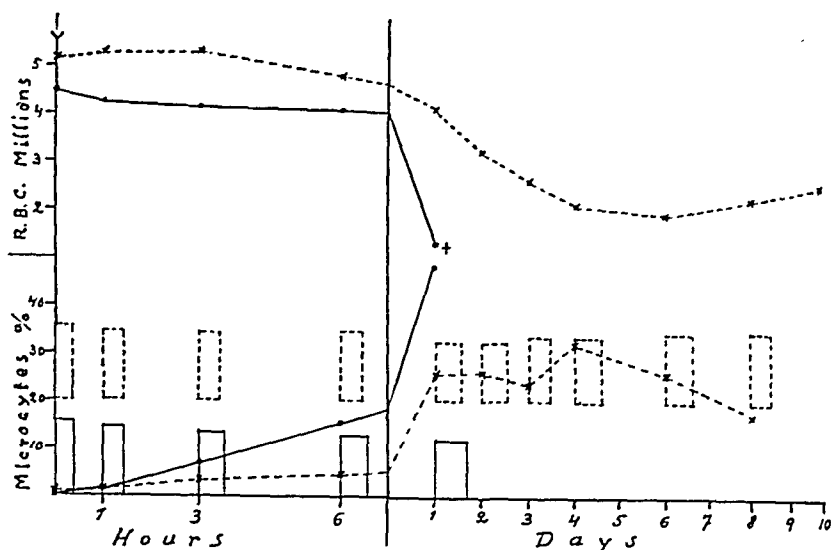


Diagram 2: Changes in the red blood corpuscle count and shape following intracardial hemolysin injections into a normal guinea-pig and into a splenectomized guinea-pig. Dose 0.125 cmm per 100 g of the titer 1 : 100. For explanation of signs see Diagram 1.

initial value of the red blood corpuscles being proportionately low, and to a poor general condition. The post mortem examination revealed an infection of the operation wound and adhesions of the intestines. Also in these experiments the spherocytosis was proportional to the hemolysis, not direct to the hemolysin dose, *i. e.* more pronounced in the normal than in the splenectomized animals.

The Action of Hemolysin in Vivo and in Vitro.

For the purpose of comparing the effect of hemolysin in vivo and in vitro it is of course necessary to be acquainted with the exact amount of blood in which the respective doses may be influential in vivo. On that account the blood volume of seven guinea-pigs was determined. The values obtained varied between 1/7.0 and 1/9.4 of body weight. The intracardial hemolysin doses given were accordingly about 0.04—0.16 ml per 10 ml blood. As the titer was 1 : 100 the doses were thus somewhat above or somewhat below the titer limit in vitro.

Three guinea-pigs, Nos. 12, 13, and 14 in Table 3, whose blood amounts were determined beforehand, were given one intra-

The effect of intracardial hemolysin injections on the red blood corpuscles. d = days, h = hours. Notation as in Table 1.

Table 3.

No.	Dose/100 g	Eryth/cmm		Mean diam.		Microcytes		Mean thickness		Notes
		Initial	Min.	Initial	Min.	Initial	Max.	Initial	Max.	
5 (spleen intact)	0.05 ml	4.03 mill.	1.35 mill. (3 d)	7.43 μ	6.40 μ (2 d)	0 %	31 % (2 d)	1.8 μ	3.6 μ (3 d)	Died after 5 days
6 (spleen intact)	0.075 ml	4.57 mill.	3.55 mill. (4 d)	7.51 μ	7.23 μ (7 d)	0.5 %	4 % (4 d)	1.9 μ	2.3 μ (1 d)	
7 (spleen intact)	0.1 ml	4.88 mill.	3.17 mill. (5 d)	7.57 μ	6.30 μ (5 d)	0 %	23 % (5 d)	1.8 μ	2.9 μ (5 d)	
8 (splenect.)	0.1 ml	4.69 mill.	3.16 mill.	7.53 μ	6.90 μ	0 %	12.15 %	1.8 μ	2.5 μ	
9 (spleen intact)	0.115 ml	5.26 mill.	1.55 mill. (4 d)	7.42 μ	6.34 μ (1 d)	0 %	35.5 % (2 d)	1.8 μ	2.4 μ (4 d)	Died after 5 days
10 (spleen intact)	0.125 ml	4.51 mill.	1.35 mill. (1 d)	7.64 μ	5.85 μ (1 d)	0 %	48.5 % (1 d)	2.0 μ	3.3 μ (1 d)	Died after 36 hours. Hemoglobinemia and hemoglobinuria.
11 (splenect.)	0.125 ml	5.16 mill.	2.00 mill. (6 d)	7.70 μ	6.25 μ (1 d)	1 %	32.5 % (4 d)	1.9 μ	3.1 μ (6 d)	

Table 3. (Cont.)

No.	Dose/100 g	Eryth./cmm		Mean diam.		Microcytes		Mean thickness		Notes
		Initial	Min.	Initial	Min.	Initial	Max.	Initial	Max.	
12 (spleen intact)	0.134 ml	4.45 mill.	3.96 mill. (6 h)	7.63 μ	6.39 μ (6 h)	0 %	18 % (6 h)	1.7 μ	2.3 μ (6 h)	Died after 20 hours. Hemoglobinemia and hemoglobinuria.
13 (spleen intact)	0.14 ml	4.09 mill.	1.46 mill. (4 d)	7.72 μ	5.98 μ (3 d)	0 %	48 % (3 d)	1.8 μ	3.6 μ (3 d)	Died after 7 days
14 (splenect.)	0.14 ml	4.41 mill.	1.75 mill. (3 d)	7.52 μ	6.59 μ (1 d)	0.5 %	22 % (1 d)	1.7 μ	2.8 μ (3 d)	
15 (spleen intact)	0.15 ml	4.27 mill.	1.31 mill. (1 d)	7.44 μ	5.49 μ (1 d)	1.5 %	59 % (1 d)	2.4 μ	4.5 μ (1 d)	Died after 30 hours. Hemoglobinemia and hemoglobinuria.
16 (splenect.)	0.15 ml	5.26 mill.	2.96 mill. (3 d)	6.95 μ	5.85 μ (1 d)	1.5 %	36.5 % (1 d)	2.3 μ	3.0 μ (1 d)	
17 (spleen intact)	0.2 ml	6.33 mill.	1.34 mill. (2 d)	7.58 μ	6.36 μ (1 d)	0 %	15.5 % (1 d)	1.7 μ	2.4 μ (2 d)	Died after 2 1/2 days.
18 (splenect.)	0.2 ml	5.69 mill.	1.77 mill. (2 d)	7.55 μ	6.46 μ (2 d)	0 %	26.5 % (1 d)	1.6 μ	2.4 μ (1 d)	Died after 2 1/2 days. Infection.

cardial hemolysin injection of 0.1 ml per 10 ml blood of the titer 1:100. No. 14 had earlier been splenectomized and the spleen of the two others was intact. As seen from the Table all animals acquired severe anemia accompanied by spherocytosis. The red blood corpuscle count of the splenectomized animal decreased 2.66 millions in three days and remission set in. The other two animals reacted very differently although the dose, in relation to the amount of blood, was the same. One of the animals, No. 12, acquired a severe hemolytic anemia and died twenty hours later. No. 13, on the other hand, did not seem to react at first more than did the splenectomized animal but died in seven days, while the last-mentioned animal recovered. We thus see that when immune hemolysin of the titer 1:100 is injected direct into the blood the dose 0.1 ml per 10 ml blood always causes a severe hemolysis *in vivo*.

Accordingly, in these tests the hemolysin was diluted about 1:100 in the animal's blood, in other words, to the titer limit. This denotes that the corresponding hemolysin concentration *in vitro* gives rise to a weak, but distinct hemolysis in the conditions prevailing at titration. A 2 per cent suspension of blood corpuscles in sodium chloride solution was however used, the final erythrocyte concentration in the tubes being only 1/3 per cent.

As stated by Ponder (17) the action of hemolysin is weaker in high blood corpuscle concentration. In addition, the normal serum inhibits the hemolysis. For comparison of the effect of hemolysin *in vivo* and *in vitro* whole blood has, on that account, to be used. The following tests *in vitro* were made:

1. Heparin blood was centrifugated and the plasma exchanged for fresh normal serum. When the blood had been mixed, 2 ml was put into two tubes. A quantity of 0.02 ml hemolysin of the same kind and concentration as used in the animal tests was added and to the second tube was added the same amount of sodium chloride solution. The blood corpuscle concentration was somewhat less than 4 millions/cmm. The tubes were placed in a water bath of 37° C for twenty-four hours. In three hours there was no hemolysins and no spherocytosis. In twenty-four hours there was strong hemolysis in both tubes. It was, however, even stronger in the control tube and this was, consequently, a non-specific hemolysis.

2. In the following test a suspension of washed blood corpuscles in sodium chloride solution with 2 % guinea-pig serum as complement

was used. The concentration was only about 2.5 million Eryth./cmm. In this case distinct hemolysis was stated three hours later and the blood corpuscles were decreased by 160,000/cmm. This was probably a specific hemolysis as there was none in the control tube. The cell thickness increased in three hours from $1.7\ \mu$ to $1.9\ \mu$. In twenty-four hours hemolysis was observed also in the control tube.

3. The non-specific hemolysis in these two tests was probably due to separation of serum and blood corpuscles (2, 10). For the purpose of avoiding this occurrence the tubes, in the third test, were placed in a slowly rotating apparatus prohibiting sedimentation of the blood corpuscles. As in Test 1, a serum suspension of the blood corpuscles was used. The test was carried out in a thermostat at 37°C . No hemolysis, and no increase in the thickness of the blood corpuscles was stated still twenty-four hours later.

We thus find a hemolysin amount which *in vivo* can dissolve all, or the majority of blood corpuscles has no effect whatsoever on whole blood *in vitro*. If, instead of whole blood a suspension of blood corpuscles in physiological NaCl solution is used hemolysis can be stated in three hours, but it is much weaker than *in vivo*. It cannot be observed later, in this case due to non-specific hemolysis setting in. We know, however, that the influence of hemolysin *in vitro* is in general terminated in two hours (15).

Discussion and Conclusions.

Ponder (17) has shown that conditions *in vivo* inhibit, rather than promote the effect of hemolysin. While the direct effect of hemolysins on red blood corpuscles is weaker *in vivo* than *in vitro*, the case is reversed if the final fate of the blood corpuscles in the organism and the tube are compared. As stated in the tests with whole blood, a quantity of hemolysin which *in vitro* cannot even affect the shape of the blood corpuscles, still less dissolve them, may, *in vivo*, bring about a severe hemolytic anemia, causing death in some cases. This can probably only be explained as an influence of the organism, and the fact that hemolysin *in vivo* has a fairly slow and prolonged course, supports the conception.

How does the organism act in this case? There is a possibility that the hemolysin is activated by the organism. In that case the test-animal's serum at the peak of hemolysis should have a strong hemolytic activity *in vitro*. This was studied in

Tests 10 and 15 (Table 3). The two test-animals died somewhat more than twenty-four hours after the hemolysin injection of violent hemolytic anemia accompanied by hemoglobinemia and hemoglobinuria. The serum could not, however, hemolyze blood corpuscles in vitro. In undiluted citrate blood weak autoagglutination in room temperature was stated in Test 15. It was however so weak that a routine titration both in the cold and in $+ 37^{\circ} \text{C}$ gave negative results in all dilutions.

In consequence, there can be no question of activation of the hemolysin in vivo — there must be a different mechanism. Probably small amounts of hemolysin which have not the power to dissolve blood corpuscles may, in some way, affect («sensitise») them so as to facilitate dissolution by the organism at a later date.

We may ask, when and where in the organism does the dissolution take place. Attention is as a matter of course drawn to the spleen. We know that familiar as well as acquired hemolytic jaundice is cured by splenectomy. Furthermore, all the animals in my tests which died of hemolytic anemia had an enlarged spleen. An analogous observation was made by Dameshek and Schwartz (7). In my parallel tests with normal and splenectomized animals the hemolysis was weaker in the latter. The difference varied however, being sometimes very great, sometimes insignificant. As the series is too small definite conclusions cannot be drawn, but as Banti (1) and others (18) have arrived at similar results, it may be stated that the effect of immune hemolysin in vivo is weaker if the spleen has been removed earlier. In any case it is more active also after splenectomy than in vitro. We may thus draw the conclusion *that the organism rapidly dissolves the red blood corpuscles which have been «sensitised» by small amounts of hemolysin and that the spleen, in this case, plays a great, yet not an entirely dominating rôle.*

Before the blood corpuscles are dissolved by the hemolysin their shape is affected in that they become more spherical. Spherocytosis may thus be considered a pre-stage to hemolysis (2, 8). In my tests it was observed that also the spherocytosis is more pronounced in vivo than in vitro and proportional to the hemolysis, i. e. it is in general stronger in normal than in splenectomized animals. In tests with whole blood in motion no change in shape of red blood corpuscles was observed in connection with the influence of such hemolysin concentrations which in vivo always bring about hemolytic anemia accompanied by spherocytosis.

When the hemolysin affects the erythrocytes in vivo the spherocytosis is, consequently, a result of the influence of the organism on »sensitised» red blood corpuscles as well — it is not a result of the influence of the hemolysin alone.

The question remains whether this »sensitisation» is identical with agglutination. The agglutinin titre was, as mentioned, much stronger than the hemolysin titre in Dameshek's and Schwartz's, as well as in my own experiments. Agglutination did not, however, occur in the tests with whole blood in vitro. In one of the test-animals a slight autoagglutination was observed. It is not quite improbable that the blood corpuscles in these tests have a somewhat increased liability to agglutinate and that this may contribute towards an earlier hemolysis. The investigation described does not, however, give an answer to this problem to which no particular attention was paid in the tests.

Summary.

Immune hemolysin was produced by immunization of a rabbit with guinea-pig blood corpuscles, and injected in variable doses into guinea-pigs. Repeated intraperitoneal injections or single doses direct into the blood were given. Following intervals of various length the red blood corpuscles were enumerated and the diameter and thickness of the erythrocytes determined. The amount of blood was measured in some test-animals. The effect of a similar hemolysin concentration in vivo and in vitro was next compared. Seven guinea-pigs had earlier been splenectomized.

Results: 1. The effect of immune hemolysin is much stronger in vivo than in vitro. 2. It dissolves a greater number of red blood corpuscles in normal than in splenectomized animals. 3. The spherocytosis is proportional to the hemolysis, *i. e.* it is more pronounced in normal than in splenectomized animals and much stronger in vivo than in vitro.

In consequence, the conclusion may be drawn that the living organism rapidly dissolves blood corpuscles influenced by small amounts of hemolysin, even if so small that they cannot directly hemolyze blood corpuscles or even affect their shape. The spleen is a great, yet not an entirely dominating factor in the capacity of the organism to hemolyse these »sensitised» red blood corpuscles.

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The Social Importance of Rheumatic Diseases in Sweden.¹

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During recent decades rheumatic diseases have been the object of ever-increasing interest in Sweden, as in most culture countries, and growing attention has been devoted to their great socio-medical significance. Kahlmeter was the first in this country to try to arrive at a numerical conception of the significance of rheumatism for invalidism by means of an investigation (published in 1923) into the causes of disablement among the material of the Pensions Board for the year 1918. His work attracted great attention, not only in Sweden but also in other countries.

When trying to establish the extension of rheumatic diseases, we have to distinguish between the number of persons falling ill annually, and the number of people suffering from these diseases at a certain point of time. The second figure depends upon the first and upon the duration of the illness in the different cases. The investigations hitherto made in Sweden have not always kept this distinction clear. The most comprehensive of these investigations was made in 1943 by a commission of experts appointed in 1941 to investigate the question of developing and augmenting the possibilities of medical care. By calling for information from all doctors who attended rheumatic patients during 1943, figures were obtained of the frequency of rheumatic diseases, and their distribution according to sex and age over the population. The

¹ While preparing this lecture the author had the advantage of consulting Professor Gunnar Dahlberg, for which he proffers his warm thanks.

Table 1.

Number of Rheumatisants who Sought Medical Treatment in Sweden in 1943, According to the Investigation of the Royal Commission on Rheumatic Diseases of 1941.

	Rhe- matic fever	Rheu- matoid arthri- tis	Degen- erative arthri- tis	Total	Scia- tica myal- gia	Total A+B+C +D
Total number	7,995	16,004	10,998	34,997	26,034	61,031
% of population	1.2	2.5	1.7	5.1	4.0	9.1
Number in need of hospital care	5,461	9,162	4,679	19,302	8,907	28,209
% of population	0.8	1.4	0.7	2.9	1.4	4.3

actual frequency of rheumatism must, however, be greater than these figures indicate. As the experts point out, on the whole the investigation accounts for the more severe cases of rheumatic disease, as those only slightly affected seldom seek medical advice. Further the reports were sometimes incomplete, as only two-thirds of the doctors who were approached replied. From this investigation it emerges (as is shown in Table 1) that during 1943 about 35,000 people sought medical care for rheumatic fever, rheumatoid arthritis, and degenerative affections of the joints, which makes 5.4 per mille of the average population of Sweden. Of these nearly one half (2.5 per mille) had rheumatoid arthritis. Somewhat less than a fourth (1.2 per mille) suffered from rheumatic fever, and the rest (1.7 per mille) had degenerative arthroses. If we also include sciatica, myalgia, and similar conditions, the aggregate figure rises to over 60,000, which is 9.4 per mille of the population. Here it may be pointed out, that figures in relation to the total population are of rather restricted value, as they are dependent on the distribution of the age groups within the popula-
tion. It is also evident that the social significance of the occurrence of the illness is largely determined by the age of the sufferer. Illness in an old man is not, of course, of the same social significance as it is in the case of a man who is still able to work, and therefore it is regrettable that due weight has not been attached to the question of age in the investigations already made.

In the investigation a distinction has also been made between those cases which called for hospital treatment and those which did not, that is to say between the more serious and the slighter cases. Of the 5.4 per mille of those suffering from rheumatic dis-

eases, 2.9 per mille were in need of hospital treatment. Thus more than half the people who sought medical advice for rheumatism were then so seriously ill that hospitalization was called for. Rheumatoid arthritis cases were most in need of treatment. In all morbidity statistics we find it difficult to delimit the disease. There are always a number of mild cases where it is debatable whether they should be included or not. When it is desired to form an opinion as to the social significance of a disease, mild cases which only cause inconsiderable or brief inability to work should naturally not be included.

An investigation along other lines, a so-called field investigation, was carried out by Edström during the years 1943—45. He let medical students examine the population of certain parts of the country in respect of rheumatic affections. In all about 72,000 people were examined. He then discovered that 38 per mille of the population suffer from rheumatic diseases in the form of rheumatic fever, rheumatoid arthritis, degenerative diseases of the joints, of myalgic conditions, etc., which were such as to impair the ability to work. Excluding sciatica, myalgia etc., the figure arrived at was 29 per mille. Thus this investigation resulted in a frequency figure for rheumatic affections which was many times greater than that arrived at by the comprehensive investigation of the above-mentioned commission of experts. The discrepancy will be due partly to doubtful diagnoses, and partly to the fact that slighter cases were also included, which makes this investigation misleading from a social point of view.

It might also be mentioned that in 1941 Ljungdahl found 21 cases of rheumatic trouble in Malmö among 1,000 recipients of sick-relief. This investigation took also account of slight cases, and above all cases of myalgia, that is cases with very doubtful diagnoses. Otherwise this material should be very valuable, as it comprises chiefly people of working age.

On the basis of the investigations already carried out, one does not, on the whole venture to say more than that it can be estimated that about 35,000 of the population of Sweden suffer from rheumatic fever, rheumatoid arthritis, and degenerative diseases of the joints, 19,000 of these being sufficiently ill to need hospital treatment.

To get a clearer idea of the situation figures for the distribution over sexes and age groups are necessary. Rheumatic fever and degenerative diseases of the joints occur about as often in men as

Table 2.
Sex Distribution in Rheumatoid Arthritis.

Investigation by	No. of cases	% women
Kahlmeter (1927)	926	60.5
Edström (1936)	11,605	54.2
Bohman (1944)	1,130	74.9
Royal commission on rheumatic diseases (1945) ..	16,004	66.8

in women. But in most investigations rheumatoid arthritis is found to be far more common among women. A glance at the survey by the commission of experts already mentioned indicates that 47.8 per cent of the cases of rheumatic fever are men and 52.2 per cent are women. Exactly the same figures are to be found for degenerative diseases of the joints. But rheumatoid arthritis shows a predominance of cases among women, who account for 66.8 per cent of the total. For the sake of comparison, one or two other investigations may be mentioned. In a work by Kahlmeter, published in 1927, it was also established that the frequency of rheumatoid arthritis was greater among women, who accounted for 61 per cent of the cases; and some years ago, in a similar investigation, the present writer found a still higher frequency figure among women, viz. 75 per cent. On the contrary, Edström in a bigger survey, which he produced in 1936, finds only a small predominance for women, viz. 54 per cent (Table 2).

To arrive at an idea of the importance of the age factor, we ought really to have figures which could be set in relation to the total population, so as to obtain figures showing the risks of falling ill at different ages, or the risks of being ill, which of course are two different things. However, the only age statistics available which refer to cases of illness are not set in relation to the whole population, and therefore they do not afford a very clear picture. The work of the commission of experts shows that the maximum incidence of rheumatic fever is between 20 and 25 years. Rheumatoid arthritis is more evenly distributed over the age groups, with the greatest frequency at 50—55 years, and cases of degenerative disease of the joints exhibit a maximum at between 55 and 70 years. These figures show the age groups of the patients of an average clientèle who sought medical advice for rheumatism during the course of a certain year. But they do not throw any light on the beginning of the rheumatic diseases or on the fre-

quency at different ages. The division into age groups indicates, however, that it is especially people of working age who are victims of rheumatism and consult a doctor.

In Sweden every citizen receives a pension from the state at 67 years of age. If a person under 67 years becomes permanently unable to work, he also gets the pension. Special taxes are imposed for these pensions, which are in the hands of a special department, the Pensions Board, set up in 1913. By scrutinizing the archives of the Pensions Board it is possible precisely to map out the invalidism within the country. As was pointed out at the beginning of this paper, Kahlmeter was the first to determine by this method the invalidism caused by rheumatism. He investigated more than 14,000 cases which were pensioned during 1918. 10 years afterwards a special committee made a similar investigation on about 18,000 cases, pensioned during the first half of 1928; and in 1944 the statisticians of the Pensions Board made a third investigation of the same kind, which is the latest one available. It comprises more than 12,000 people who had been pensioned that year. It must be observed, however, that these pensioners were all under 67 years of age, which was not the case in respect of the two earlier investigations, which included older people. (Table 3 gives a survey of these investigations.) We find that old-age diseases (arteriosclerotic affections etc.) come first among the causes of disability in all three investigations (56.3, 43.5 and 25.6 per cent respectively). That the percentage is considerably lower in the survey of 1944 than in the earlier surveys, is of course due to the fact that it does not embrace the age groups above 67 years. The next most important causes of disability are the rheumatic diseases (rheumatoid arthritis and degenerative disease of the joints) — 9.1, 12.4, 16.8 per cent respectively. For the sake of comparison, it may be mentioned that invalidism due to pulmonary tuberculosis is much less, being about 50 % of the figures quoted. Of course the different surveys are easier to compare if we eliminate the diseases due to old age. Among the remaining causes of invalidism we find that rheumatic diseases take first place, with somewhat more than 20 per cent in all investigations, figures which are remarkably close to each other, and which might indicate a similar rheumatic invalidism in all three surveys. This applies to chronic rheumatoid arthritis and degenerative arthrosis. We do not find any permanent disability after rheumatic fever, as the cases which result in invalidism are looked upon

Table 3.

Investigations as to the Causes of Invalidism in the Pensions Board Material.

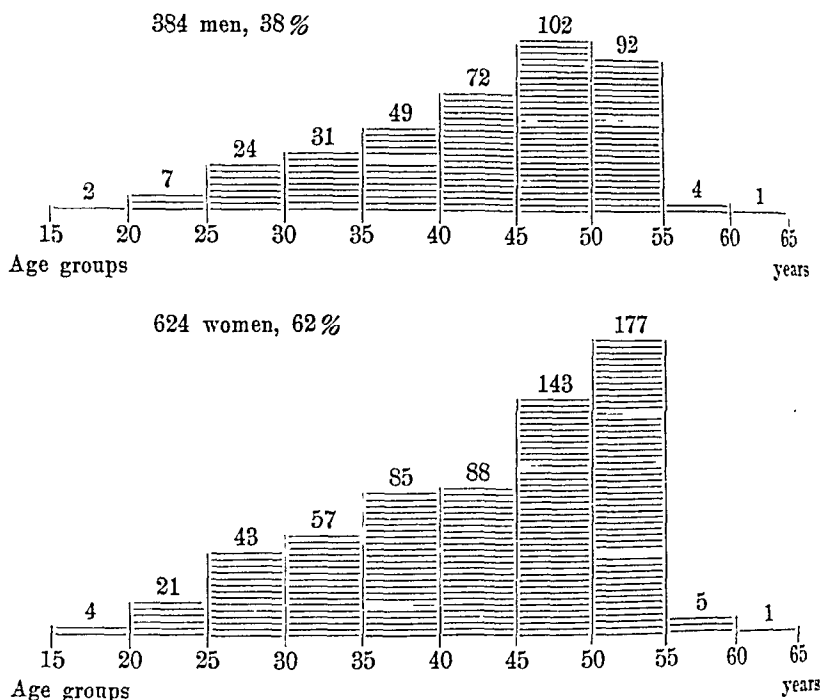
Cause of invalidism	The investigation of 1918			The investigation of 1928			The investigation of 1944		
	14,607 persons pensioned in 1918			18,055 persons pensioned during the first half of 1928			12,452 persons under 67 years pensioned in 1944		
	Number of persons	% of the whole material	% of the material minus old-age diseases	Number of persons	% of the whole material	% of the material minus old-age diseases	Number of persons	% of the whole material	% of the material minus old-age diseases
Old-age diseases ..	8,241	56.3		7,854	43.5		3,190	25.6	
Rheum. arthrit. and degen. joint disease	1,331	9.1	20.9	2,245	12.4	22.0	12,092	16.8	22.6
Pulmonary tuberculosis	852	5.8	13.4	976	5.4	9.6	1,010	8.1	10.9
Valvular defects ..	348	2.4	5.5	731	4.1	7.2	449	3.6	4.8
75 % of valvular defects	261	1.8	4.1	548	3.0	5.4	337	2.7	3.6

as chronic. However, the affections of the heart which are caused by it, very often result in a serious reduction in the capacity for work. In the 1944 material we find, for instance, 449 persons or 3.6 per cent who are invalids owing to valvular defects. It is difficult to decide to what extent these have a rheumatic origin, but most authors state that 75 per cent of all valvular defects are caused by rheumatic fever, and if we accepted this no doubt very unreliable figure, then 337 persons, or not quite 3 per cent of the last mentioned category would be invalids as a result of rheumatic heart defects. Thus one can claim that rheumatic diseases cause more than 1/5 of the total invalidism, when the affections resulting from old age have been eliminated. We could also deduce directly from the investigations the annual number of people who become invalids as a result of rheumatism. In 1944, for instance, there were about 2,500 people under 67, which makes about 0.4 per mille of this part of the population. It would also be of great socio-medical interest to establish how many rheumatic

¹ Thereof: Rheum. arthrit. 989.
Degen. joint disease 1,103.

Table 4.

Age Distribution of Rheumatisants Invalids According to an Investigation by F. Sundelin 1942.



invalids there are in the country, but it has been found impossible to calculate this exactly, although different methods have been tried. Thus Kahlmeter estimated the number at 60,000 in 1918. On the basis of the official statistics which were available in 1936, Sundelin calculated that by 1942 about 42,000 persons would be prematurely pensioned on account of rheumatic diseases. However, if we only consider cases under 67 years of age, it is possible to reach a more exact figure. We know that in 1943 there were 153,000 invalids under 67 years. According to the investigation in respect of invalids in 1944 which has already been described, rheumatism, inclusive of a certain number of valvular defects, accounted for about 20 per cent of the causes of disability. Therefore at present the number of rheumatic invalids under 67 will probably be at least 30,000, which is about 5 per mille of the population under 67.

To make the picture more complete, we can emphasize some details. In a work published in 1943 which deals with more than

1,000 cases, Sundelin elucidated the question of rheumatic disability from various aspects. It emerges that a great many more women than men are disabled as a result of rheumatic disease (62 per cent women). As regards the ages of the rheumatic invalids this investigation shows that the greatest number of men are between 35 and 55, while the greatest number of women are between the ages of 25 and 55. This might indicate that women fall victims to disabling rheumatoid arthritis at an earlier age than men (Table 4). In this material, too, the worst cases more often appear in women, which points in the same direction. As regards the degree of disability we must of course take into consideration that a number of those who receive pensions on account of rheumatism are still capable of doing some work. The investigation establishes that about one third of the men are able to do some remunerative work, while only very few of the women are. Sundelin points out that the State pension for invalids is not at present enough for the full support of the recipient but further help from the local authorities or from other sources is necessary. Hence it follows that at least $\frac{2}{3}$ of the men and most of the women have to get such help (1947).

To arrive at a more complete conception of the social significance of rheumatism we must also consider its mortality rate. Rheumatic fever is of very considerable significance as a cause of death, though the disease in Sweden is only directly responsible for about 20—50 deaths yearly. However, the figures which are to be found in official statistics are not compiled in such a way as would enable us to venture on even approximate calculations. It might be pointed out that chronic heart diseases caused 1,595 deaths under the age of 60 in 1943. To what extent these were due to rheumatic valvular defects it cannot possibly be decided. The corresponding number of deaths above the age of 60 were 5,893 and it may be taken that arteriosclerosis played a predominant part. With regard to the previous statements we might perhaps dare to maintain that each year probably more than 1,000 people die as a result of a rheumatic heart. The number of deaths caused by pulmonary tuberculosis which is usually mentioned for comparison is nearly 4,000 per annum.

The figures adduced will give some idea of the losses to the country due to rheumatism, in the form of illness, disability, and premature death. It may be asked what these diseases cost the country. It is usual to calculate the number of working days lost

Table 5.

Losses due to Rheumatism in Sweden.

	Working days lost	Cost of financial aid (in swedish crowns)	
		Disablement pensions	Other aid.
Rheumatic invalidism	9 mill. (max.)	21 mill.	10 mill.
Rheumatic affections	5 mill.		20 mill.
Total	14 mill.	51 mill. sw. cr.=14 mill. \$	

and the earnings lost as a result and regard this sum as the total loss. It should however be taken into account that if a person is working and earning money, then he is also spending, and his spendings might exceed his earnings. It is also very difficult to estimate the number of working days lost, as they depend on the age distribution of the cases. For instance, that a child is unable to work, is of course of no economic importance. As already mentioned, the rheumatic invalids of working age probably amount to about 30,000 people. If we count 300 working days for each individual, this would mean a loss of 9 million working days each year. However, to what extent this is a loss to society, is difficult to decide, since we cannot estimate how much the production of the person in question exceeds his consumption. In either case we cannot count loss of working days as well as maintenance payments to the sick person but only one of the two. Owing to the reduction of their ability to work, about 21 million Swedish kronor has to be paid annually by the Pensions Board to those disabled by rheumatism. It is impossible to decide in what proportion this money represent compensation for work not performed and in what proportion it is a contribution for medical care. So these disbursements too, are difficult to evaluate. At least 20,000 of the disabled are quite unable to make any contribution to their own support, and it costs at least 10 million Swedish kronor in addition to their pensions to maintain them, reckoning with 1,200 Sw. kr. as the minimum of subsistence, which is of course a low estimate (Table 5).

Also it is difficult to judge what losses the rheumatic diseases involve for the country. The 1941 commission of experts, basing their investigations on the records of sickness relief, found that at

least 5 million working days are lost as a result of occasional inability to work owing to rheumatic disease. Calculating with the same minimum of subsistence as above, at least 20 million Sw. kr. are required for the maintenance of those suffering from rheumatic illness. This sum cannot, of course, cover all expenses, nor provide for the support of the family of a sick person, etc. In all, rheumatism must cause a loss of 14 million working days and cost the country in provision for maintenance etc., at least 50 million Sw. kr., or about 14 million dollars at the present rate of exchange. For the sake of comparison, it may be mentioned that the total State income in 1946—47 was about 3 milliards of Sw. kr.

Table 6.

Result of Treatment of Rheumatic Arthritis at the Pensions Board's Hospitals, According to Investigations 3 Years Last Treatment.

Investigation by	No. of cases with rheum. disease.	% capable of work
Kahlmeter (1923).	428	58.9 %
Kahlmeter (1925).	547	64.9 %
Alderin (1927) ...	257	55.3 %
Bohman (1942) ..	1,654	66.4 %

What then has been done in our country to combat the rheumatism diseases? Most of the sufferers are naturally being nursed in our hospitals and cottage hospitals. But a few special hospitals have also been established. These are the result of the initiative of the Pensions Board, which since 1915 has been responsible for the care of the sick. Thus the Pensions Board now has 3 independent medical institutions, which in part are occupied by patients suffering from rheumatic diseases, 6 special wards for rheumatism at other hospitals, and some beds at certain summer health-resorts. The total number of beds amount to about 580. About 3,600 patients are nursed at these hospitals every year, the majority of these patients suffer from rheumatoid arthritis (in 1946 the figures were 1,193 men and 2,468 women). The results of this work can be deduced from several post-examinations, 2 made by Kahlmeter in 1923 and 1925, and the last one made by myself in 1942 (published in 1944). It appears from these that about 60 per cent of the patients who have received treatment through the agency of the Pensions Board are quite or almost

¹ 8.5 % were partially capable of work.

quite able to work about 3 years after the last period of care. (Table 6). However, in spite of this, it has been shown by several investigations by experts in the field, the last being the commission of 1941, that the possibilities of treatment for rheumatic patients are insufficient. Far-reaching measures have been proposed, but none have yet been realized.

Conclusions.

As a final summing up there are some points to be particularly emphasized, namely:

that at least 35,000 people per annum contract rheumatic diseases which call for medical care — some of them are ill only for a short time,

that rheumatism accounts for about 20 per cent of the invalidism, if old-age diseases are excluded,

that every year about 2,500 people are prematurely pensioned owing to rheumatism, and that there are at present at least 30,000 rheumatic invalids of working age in the country,

that probably more than 1,000 people die each year as the result of rheumatic heart-failure,

that rheumatism is responsible for 14 million working days lost to the country every year, and

that the cost of the maintenance etc. of those suffering from rheumatic diseases amounts to at least 14 million dollars.

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Some Experiments on the Treatment of Hemophilia with Mercury.¹

By

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(Submitted for publication January 30, 1948.)

In the winter of 1945—46 I observed the occurrence of multiple thromboses in a cardiac patient who was receiving repeated injections of a mercurial diuretic (Injectio Mersalyli B. P.) at frequent intervals for his edema. Re-examination of some other patients treated with the same preparation showed that two others had had thromboses during treatment. The question arose as to whether the mercurial diuretic used was responsible for the thromboses. If this were the case the simplest explanation would be that the preparation might shorten the clotting time of the blood.

As the more refined methods of estimating the clotting times were not immediately available I tested the effect of preparations of mercury on several hemophiliacs, in whom tests of this sort give so much more dramatic results than in persons or experimental animals with a normally short clotting time. The determinations were made at room temperature both by direct inspection of the course of the clotting in samples of blood taken in a wide glass tube and by Hedenius' (1936) method. Each test was carried out in duplicate.

I have had the friendly cooperation of Dr. Erik Sköld, Deputy Medical Superintendent of the Second Medical Department at St. Erik's Hospital, Stockholm. Since working on his monograph (1944) on hemophilia in Sweden he has maintained contact with

¹ Lecture delivered before the Fourth National Medical Assembly in Stockholm November 22nd 1947.

many hemophilics and several of these placed themselves at my disposal for the necessary experiments.

The first experiment (March 8th 1946) gave a negative result: the clotting time of a bleeder (B. O.) was more or less unchanged 4 and 8 hours after an intravenous injection of 1 c.c. Injectio Mersalyli. However, in the next experiment on another hemophilic (T. L.) and using different times for taking the samples, a clear positive result was obtained, as may be seen from Table I. As the investigation proceeded it became clear that some hemophilics gave a positive reaction, *i. e.* showed a shortening of the clotting time, while others did not.

Table I.

Name: T. L.

20. 6. 46	2.0 p. m.	Clotting time 74 mins.	
			1 c.c. Inj. Mersalyli was given intravenously immediately after withdrawal of the sample.	
20. 6. 46	5.0 p. m.	Clotting time 55 mins.	
21. 6. 46	10.0 a. m.	»	» 7 »
22. 6. 46	10.0 a. m.	»	» 120 »
22. 6. 46	1.0 p. m.	»	» 82 »

The table shows that a marked diminution in the clotting time occurred after an intravenous injection of Injectio Mersalyli, and that the effect was preceded by a certain period of latency. As regards the original value for the clotting time, *i. e.* that before the injection of the mercury, I should state that in this, as in all the other cases (with one exception to which I shall return) it was at a level which may be taken as the normal for the patient in question at a certain interval after the last blood transfusion, the interval being different in different cases. All the bleeders included in my series have been well-known in St. Erik's hospital for many years.

The next experimental subject (E. R.) showed the same type of reaction as the one just described.

Table II.

Name: E. R.

21. 10. 46	10.0 a. m.	Clotting time >24 hours.	
			1 c.c. Inj. Mersalyli was given intravenously immediately after withdrawal of the sample.	
		10.30 a. m.	Clotting time >24 hours.	
		11.01 a. m.	»	» 34 mins
		12.0 noon.	»	» 44 »
		2.0 p. m.	»	» 61 »
		6.0 p. m.	»	» 20 »
22. 10. 46	10.18 a. m.	»	» 20 »

On November 11th 1946 the fourth case received intravenously one ampoule of Esidrone (Ciba)/0.14 g of the sodium salt of β (theophylline-7-mercuri)- γ -hydroxypropylamido-quinolinic acid). Before the injection the clotting time was 180 mins., 9 hours afterwards it was 36 mins. and 24 hours after the injection it was 47 mins. Thus there is a definite effect in this case also.

The next case (B. P.) received 2 ml Injectio Mersalyli intravenously. No effect whatsoever was observed in this patient.

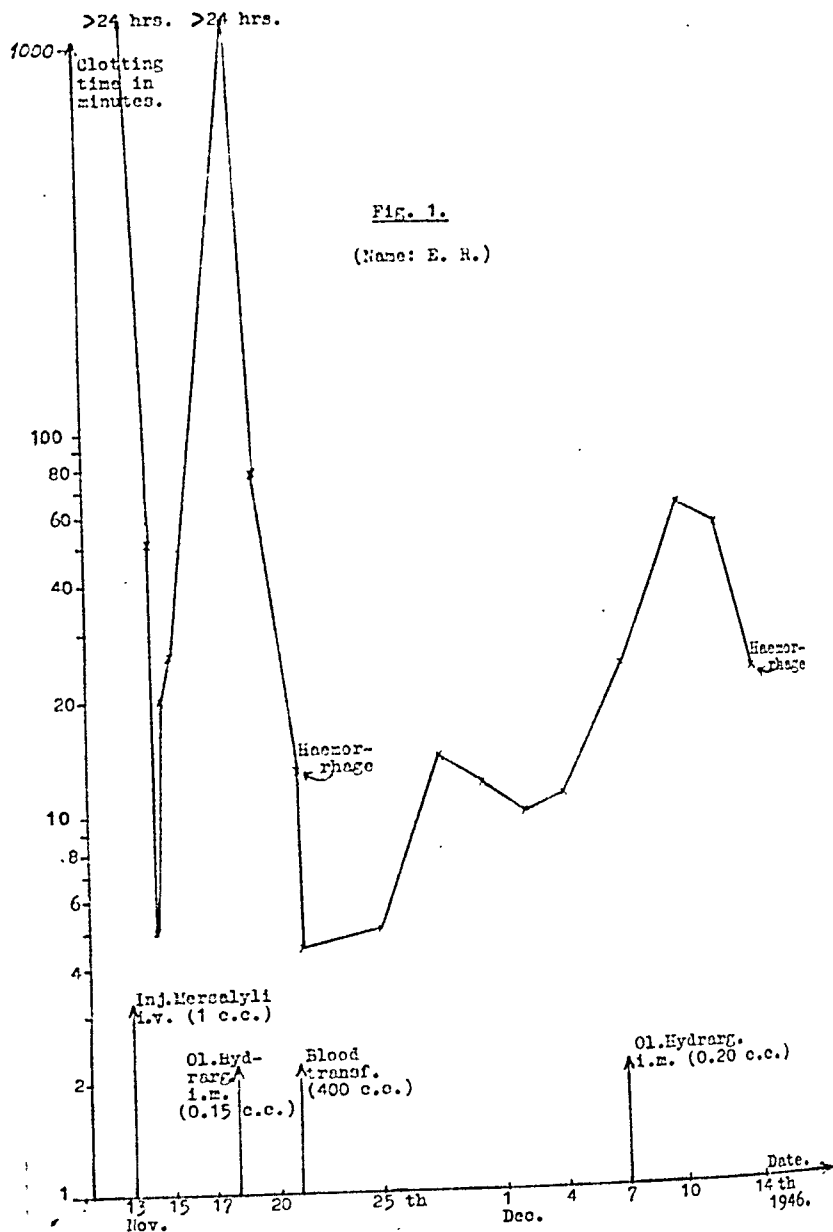
On the assumption that the mercury was the active agent in these injections some further experiments were made in which Oleum Hydrargyri (P. Svec., containing 41.75 % Hg) was injected intramuscularly in an attempt to produce a more lasting effect on the clotting time. The patient was the same who showed a positive result when treated with Inj. Mersalyli as shown in Table II. and gave another good reaction to an intravenous injection of Inj. Mersalyli. The further course of the experiment may be seen from Fig. 1.

The first thing to be seen is the large effect produced by the intravenous injection of Inj. Mersalyli. It is, however, of short duration. A test made 5 days later shows that the clotting time is maximal again. At this point the patient received 0.15 ml Ol. Hydrargyri intramuscularly. The effect on the clotting time was pronounced though slower in onset. However, in spite of the diminution in the clotting time, the patient developed a large hematoma at the site of the injection 3 days after it was administered and had to receive a blood transfusion. This gave rise to a marked diminution in the clotting time of relatively long duration. When it began to increase again the patient was given a new mercury depot. The clotting time began to decrease again after a latent period, but nevertheless a new hematoma occurred at the site of the new depot, and a further transfusion had to be given.

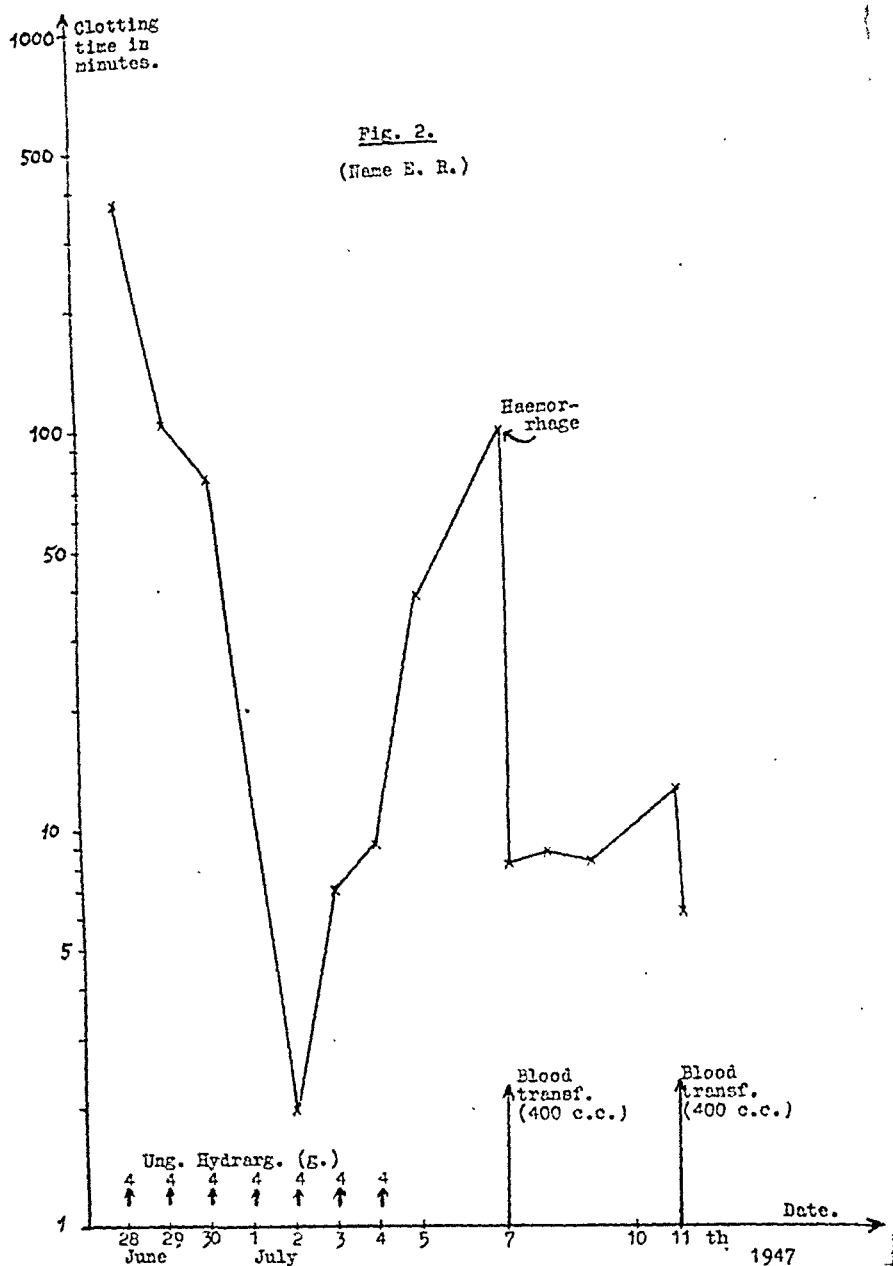
This experiment shows

- 1) That mercury can diminish the clotting time in hemophilia, or even reduce it to normal.
- 2) That even a considerable diminution in the clotting time is not enough to prevent the patients from having hemorrhages.

The clinical effect is thus not satisfactory, although it looks well from a numerical point of view. There is, however, a considerable trauma associated with the laying of an intramuscular depot of oil, and it might be held that the mercury had done some good though not enough to counterbalance this trauma. Experi-

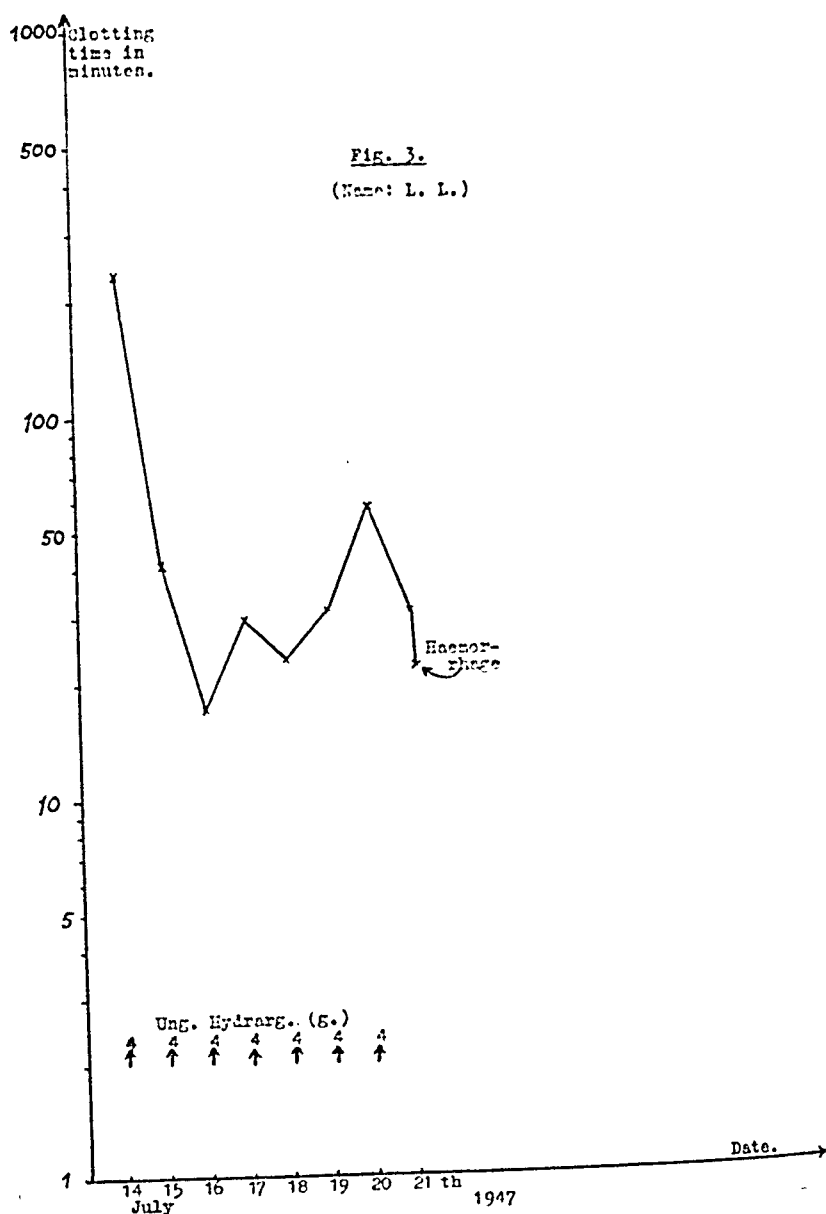


ments were therefore carried out in which the mercury was administered atraumatically, *i. e.* by inunction of Ung. Hydrargyri (P. Svec. Ed. X., containing about 30 % Hg). It is held that a very uniform rate of absorption of mercury can be achieved in this way.



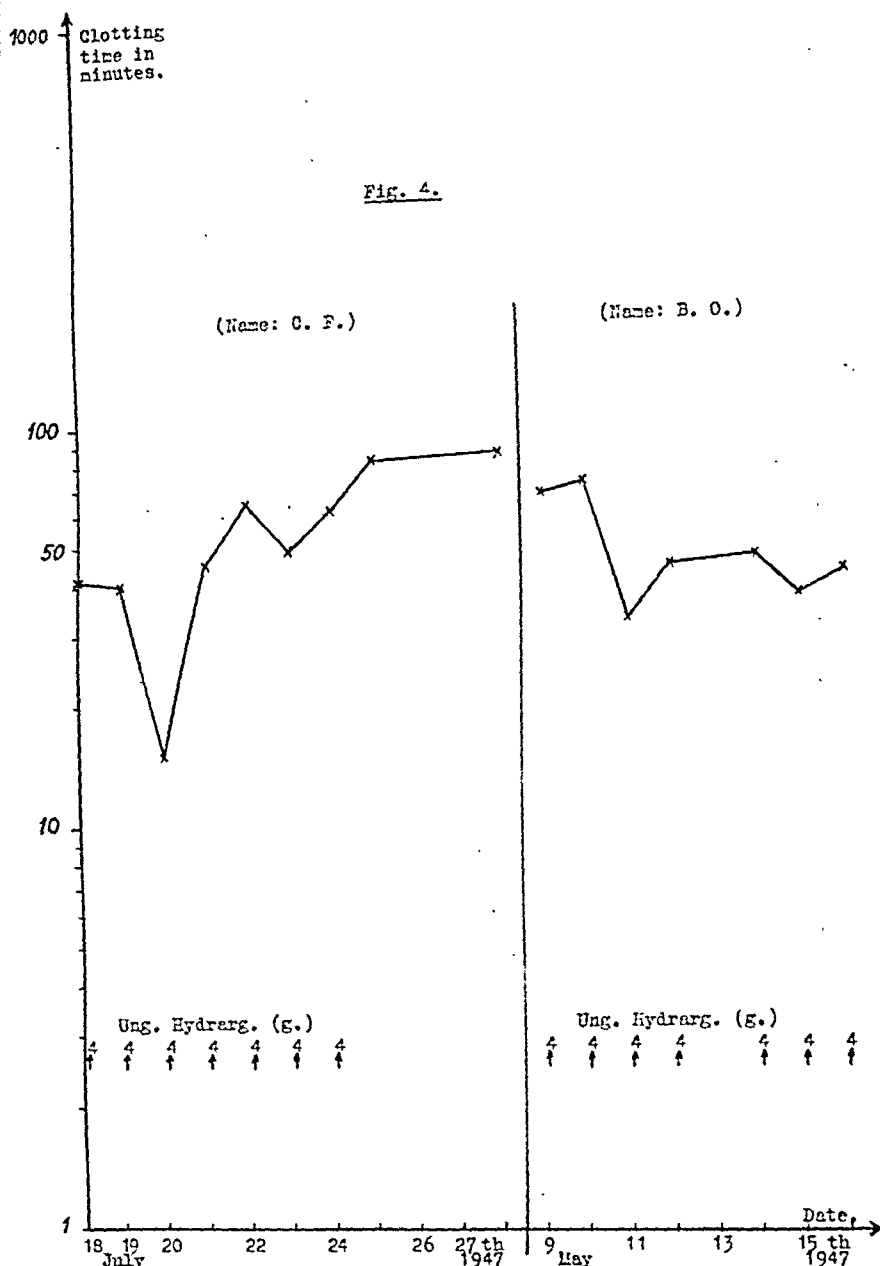
Four hemophiliacs were inoculated with 4 g Ung. Hydrargyri for $\frac{1}{2}$ hour daily for a week. A diminution of the clotting time occurred in two of them, in the other two cases there was no effect, as is seen from Figs. 2, 3 and 4.

Fig. 2 refers to the same mercury-susceptible patient who re-



ceived the intramuscular injections. It may be seen that there was a definite effect on the clotting time, but shortly after the end of the treatment the clotting time rose again and a hemorrhage into a joint necessitated a transfusion.

Fig. 3 shows the other positive case. A certain diminution in



the clotting time was achieved, but the patient had a hemorrhage almost immediately after the end of the course.

Finally Fig. 4 shows two cases in which there was no definite effect produced by the inunction of Ung. Hydrargyri. The latter of these is the person who was the subject of my first experiment,

and thus was one who did not react within 8 hours to Inj. Mersalyli given intravenously.

The reactions of all the experimental subjects have been summarized in the following table.

Table III.

Name	Mercurial Diuretic Intravenously	Ol. Hydrargyri Intramuscularly	Ung. Hydrargyri Percutaneously
C. F....			0
L. L....	+ (Esidrone)		+
T. L....	+ (Inj. Mersalyli)		
B. O....	0 (" ")		0
B. P....	0 (" ")		
E. R....	+ (" ")	+	+
" ...	+ (" ")	+	
" ...	0 (" ")		

+ = significant shortening of the clotting time.

0 = no definite effect.

The table shows that one patient (E. R.) who usually reacted very well did not react on one occasion to an intravenous injection of Inj. Mersalyli. However, at the time of this test the starting value for the clotting time was only 30 mins. though a much longer one was to be expected, and it is possible that there was some mistake in this case. One does not know whether those patients who did not react to a single test would have reacted to further tests. It is, of course, possible that they would. If not perhaps there are some cases which never react and it would be interesting to know that as well. Perhaps there are various forms of hemophilia.

So far as is known none of the bleeders included in these series are related to one another.

I cannot offer any explanation of the mode of action of the mercury in those cases where a positive reaction occurred. The addition of Inj. Mersalyli to hemophilic blood *in vitro* in dilutions of 1/100—1/10,000 did not lead to any diminution in the clotting time. There was no significant difference between the prothrombin estimations and thrombocyte counts made before and during the treatment. Neither was there any increase in fragility of

the thrombocytes after the medication as shown by counting them before and after shaking a sample of blood in a tube containing glass beads.

Since I began these experiments David I. Macht (1946) has published two papers in which he shows that the clotting time of the blood of normal experimental animals is diminished after the administration of mercurial diuretics. Thus it is not only the pathological process of clotting found in hemophilia which is affected by these preparations, and we have an explanation for the thromboses in cardiac patients treated with Inj. Mersalyli mentioned in introducing this paper.

Summary.

A clinical observation of multiple thromboses in a patient who received repeated and relatively frequent injections of a mercurial diuretic on account of edema of cardiac origin led to the testing of the effect of various preparations of mercury on the clotting time of hemophilics.

Six hemophilics were available. Seven experiments involving the intravenous injection of a mercurial diuretic (Inj. Mersalyli or Esidrone Ciba) were carried out on five of the subjects. No effect was obtained in three of the experiments. After a period of latency of several hours four of the experiments gave a significant diminution of the clotting time with a minimum at about 24 hours. After this the clotting time rose again sharply.

Two tests using intramuscular injections of Ol. Hydrargyri were performed on one of the hemophilics. These showed a diminution of the clotting time which was more gradual in onset and lasted for several days.

Four of the hemophilics were treated for a week by daily inunction of 4 g of Ung. Hydrargyri during $\frac{1}{2}$ hour. In two of them no effect was seen; in the other two the clotting time was maintained at a significantly lowered level during treatment.

The prothrombin index, thrombocyte count and fragility of the thrombocytes seem to have been unaffected by the preparations used in the dosage in which they were given.

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Studies on the Serum Proteins in Hepatitis.

III.

Serum Protein Variations in Chronic Hepatitis and the Clinical Course of the Disease.

By

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(Submitted for publication January 26, 1948.)

During the past few years, dating from 1941, there have in Denmark been very distinct epidemic waves of acute hepatitis, with a crest in late autumn, and in the same period it has been possible to record an increasing frequency of chronic hepatitis. One particular group of the population, women who have got over the menopause, are especially disposed to this latter form of hepatitis (1—5).

The behaviour of the serum proteins during the course of this disease was studied in two previous works, the first dealing with the relation between albumin and globulin (6), the second with the Takata reaction and the Gros reaction (7). In the present article I shall describe the connection between the variations of the serum proteins and the clinical course of the disease, mainly with the aid of diagrams and brief case reports.

The changes in the serum proteins have claimed the attention of several workers previously (8—14). A globulin increase and an albumin decrease had been observed in hepatitis and cirrhosis. The serum protein changes and the degree of liver damage run parallel (10). The clinical course of the liver disease follows the albumin variations. A fall of the serum albumin is a bad prognostic sign (13). The decrease in the albumin is attributed to reduced albumin synthesis in the liver. It is possible that a loss of albumin into the ascites fluid as a result of frequent paracenteses may play some rôle (8). The increase of globulin is thought to be the result of the formation of antibodies during the infection (9).

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Table 1.

Patients who recovered:

<i>Patients who recovered:</i>					
No.	Sex	Age	Months from onset to recovery	Months observed after recovery	Figure
A. Transient low albumin values, high globulin values and ascites.					
1	F	56	36	1	I
2	F	60	30	6	
3	F	39	10	5	II
4	F	42	9	7	
5	M	33	3	7	
6	M	56	6	7	
B. Normal albumin values, constant or increasing. Transient rise in globulin values.					
7	F	66	21	12	III
8	F	63	15	22	
9	M	45	7	5	

Patients who died:

Patients who died:				Months from onset to death	Figure
No	Sex	Age			
A. Albumin gradually decreasing, ascites before death.					
10	F	73	9½	IV	
11	F	55	20		
12	F	44	15		
13	F	58	8		
14	F	76	16		
15	F	36	6½		
B. Albumin gradually decreasing, no ascites.					
16	F	49	14	V	
17	F	59	3		
18	F	65	5		
19	F	41	3		
C. Albumin rising, thereafter decreasing, ascites before death.					
20	F	73	16	VI	
21	F	65	8		

I have gone further into these matters with a material of patients suffering from chronic hepatitis, the serum proteins being analyzed at regular intervals. The method employed was Henriques & Klausen's ammonium sulphate analysis (15). Double analyses were made in every case. I also employed Meulengracht's icterus index determination and the Takata reaction. The latter reaction was judged to be positive only when there was distinct precipitation in at least three successive tubes; according to the degree of precipitation I have described the result as +, ++ and +++. Table 1 and Fig. 1—6 show the results of these investigations made on 21 patients. Table 1 visualizes the material, which is made up of 18 women and 3 men. Twelve women died during the period of observation; 3 men and 6 women are regarded as recovered, *i. e.* they felt well at the time of the follow-up examination,

without tiredness and nausea, with a normal icterus index and negative Takata. The material is divided in groups according to the variations in the albumin and globulin percentages. Among the patients who recovered 6 patients had transient ascites and oedema. The albumin concentration was low during the period when the patient had these symptoms and rising when ascites and oedema disappeared. This type is illustrated by the case records from patients No. 2 and 3 (Figures I and II).

3 patients had normal albumin values, constant or increasing, while the globulin concentrations were transiently rising. This type is illustrated by the case record from patient No. 7 (Figure III).

In the group of patients who died 10 patients had a gradual fall in albumin concentration, 6 developed ascites and oedema (illustrated by case record from patient No. 11 (Figure IV)), while 4 died without these symptoms (illustrated by case record from patient No. 18 (Figure V)). In 2 patients the albumin concentration was in the beginning rising, thereafter decreasing. Ascites appeared before death in both of them (illustrated by case record from patient No. 20 (Figure VI)).

The diagrams show that considerable fluctuations occur in the concentrations of albumin and globulin. It will be seen that the fluctuations are mostly in opposite directions, low albumin values being accompanied by high globulin values, and vice versa. It is also clearly observable that the Takata reaction follows the fluctuations in the albumin: globulin ratio. Where A : G falls, Takata is positive, whereas when A : G rises it is negative. Compared with the case reports, these serum protein analyses confirm the observation that the fluctuations in the albumin concentration follow the clinical condition, lower albumin accompanying exacerbation and higher albumin an improvement in the condition. Not one patient in the material survived a fall in the albumin percentage below 2.48. When the albumin percentage is higher it is difficult to say anything about the prognosis. Four out of ten survivors had less than 3.0 % albumin at one time or another (Nos. 1, 2, 3, 4); eleven who died had less than 3.0 %. Albumin values of between 2.5 and 3.0 % do not preclude recovery.

Regarding the connection between serum proteins and the occurrence of ascites and oedema, the material¹ gives the following picture: of 6 patients without ascites and oedema, 2 had under 3.0 % albumin (No. 16: 2.47 % and No. 18: 2.77 %). One

out of 15 patients with ascites and oedema had a serum albumin concentration of over 3.0 % (No. 2: 3.3 %). In the observation period 2 men and 4 women had ascites and oedema which subsided (Nos. 1—6). Simultaneously the serum albumin fell and rose. Using figures from this material I have previously shown that when the value $2.5 \times \text{albumin}\% + \text{globulin}\%$ fell below 11, ascites and oedema set in (6). In a larger material Bjorneboe, Brun & Raaschou showed that these figures must be corrected to $3.5 \times \text{albumin}\% + \text{globulin}\%$ and 14 (16). It would thus seem that in these patients the globulin increase to some extent compensates for the albumin decrease as regards keeping the colloid osmotic pressure up above the oedema limit.

The explanation of the fall in the albumin in liver lesions is thought to be that the albumin is produced in the liver cells and that this production declines when the liver cells are damaged. In this connection I might mention that among normal individuals in Copenhagen (examined in the period October 1942—April 43) was found a group with a lower serum albumin than the average, this group consisting of women over 35 years (17). Possibly this means that the livers of these women had been damaged, which may be the reason why the prognosis for hepatitis in elderly females in Denmark during these years has been worse than in other population groups.

It is usual to attribute the increase of serum globulin to the formation of antibodies, in which connection it may be mentioned that another chronic virus infection, lymphogranuloma inguinale, shows similarly high globulin values (18).

Cataphoresis tests have shown that in hepatitis and liver cirrhosis there is generally an increase in the γ globulin in serum. Sometimes there is also an increase in the β globulin (19—25). There are antibodies in the γ globulin, and the circumstance that this globulin fraction is higher in hepatitis argues that at any rate part of the globulin increase is due to the formation of antibodies.

As I have said, high globulin concentrations are accompanied by low albumin concentrations. In an earlier work I found a linear relation between albumin and globulin concentrations (6). How-

¹ With regard only to blood samples taken on patients when in bed. Blood samples taken on ambulatory patients show higher serum protein values than blood samples taken on patients in bed owing to the concentration of the blood in the erect position. This question is discussed in 16) and 17).

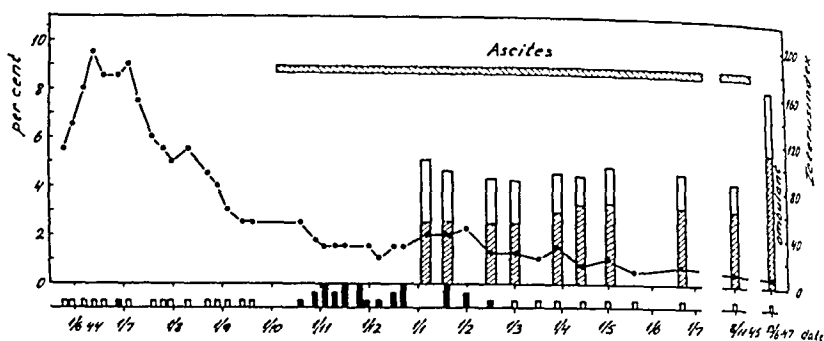


Figure I

Woman 60 years old. Duration of illness before admittance: $1\frac{1}{2}$ weeks.

— Icterus index □ Takata ÷ ■ Takata + ■ Takata ++ ■ Takata +++
 ▨ Albumin □ Globulin
 (See case record of patient No. 2.)

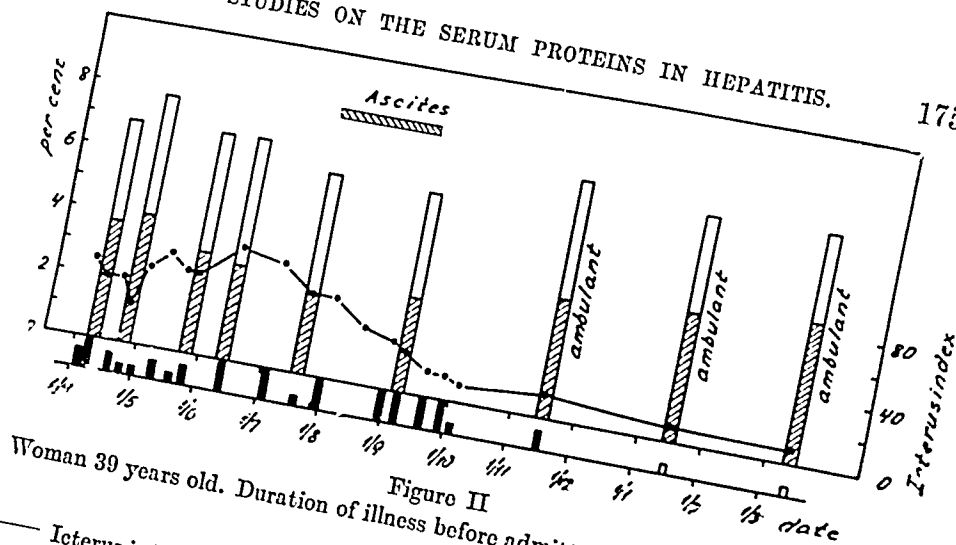
ever, in a larger material it turned out that this relation is not so simple (16). The fact that the albumin varies in a direction opposite to the globulin may be due to several causes: 1) A mechanism of regulation for the colloid osmotic pressure which regulates albumin and globulin in relation to each other (6). 2) Severe liver lesion with reduced albumin synthesis simultaneously with high antibody production. 3) Change in the relative rate of production and utilization of albumin and globulin fractions in hepatitis (24, 25). It is possible that all these mechanisms are in operation independently (Martin, 25).

Case Book 1423/44. 23-5 to 19-9-1944.
 921/45. 16-10-44 to 13-7-45.
 1596/45. 15-11-45 to 20-11-45.
 564/46. 26-3-46 to 12-4-46.

Patient No. 2, case record (Figure I).

Female, 60 years. Menopause at about 45th year. Previously well. Ten days before 1st hosp. tiredness and nausea. 4 days later jaundice. Liver not enlarged on admission. Intense jaundice. Galactose test positive (3.6 g). Liver biopsy showed widespread acute hepatitis. Patient complained of tiredness and nausea, not of gastric pain. Jaundice subsided and patient was discharged 4 months after admission (owing to disturbances in Copenhagen). A week after discharge she had ankle oedema and she observed increasing abdominal dimensions. Jaundice increased again.

At the 2nd admission 1 month after discharge there were ascites and oedema of the lower extremities and the loins. While in hosp. the patient was much troubled with ascites and oedema. She was



— Icterus index □ Takata ÷ ■ Takata + ■ Takata ++ ■ Takata +++
 // Albumin □ Globulin
 (See case record of patient No. 3.)

treated with Mersalyl and ammonium chloride, salt-free diet, a single ascites puncture. Ten weeks after the 2nd admission X-ray revealed fluid in right pleura (hydrothorax?). Seven months after 2nd adm. On discharge 9 months after 2nd adm. the principal complaint was abdominal tension. Thereafter the condition remained stationary. She had oedema and ascites, which was treated with monthly punctures and weekly injections of Mersalyl. Appetite good. On the 3rd adm. dry serum was tried on the patient, the result being a grave condition of shock, and on the 4th adm. she was given 8 blood transfusions of $\frac{1}{2}$ litre daily. With these transfusions it was possible only to bring little influence to bear on the serum colloid osmotic pressure. The serum proteins, which were followed for about 8 months after the onset of the disease, showed an increased albumin percentage and a low globulin value, but for both proteins the absolute values were low until the examination in 1947 (see below). At the same time Takata changed from positive to negative.

During the first two hosp. periods the temperature was sub-febrile, with occasional brief but marked fluctuations; later it was normal. The after-examination 14 months after the 4th discharge showed that the patient no longer had oedema or ascites. Is now quite well. The last ascites puncture was made about four months ago. The Takata reaction is normal, the serum albumin and globulin likewise.

Case Book 1357/45. 2-4 to 13-10-45.
 Patient No. 3, case record (Figure II).
 Female, 39 years. Salpingitis diagnosed six years before hospitalization. Menses formerly regular. Five months before admission was

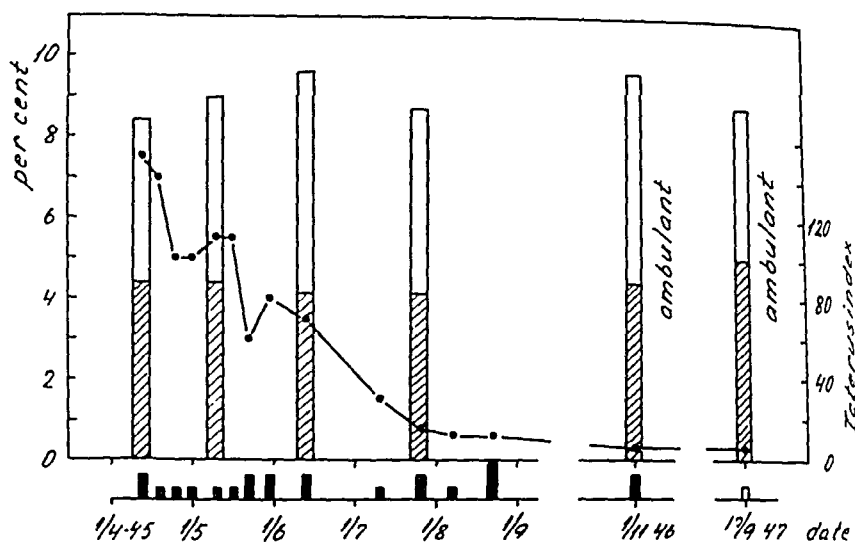


Figure III

Woman 66 years old. Duration of illness before admittance: 4 weeks.

— Icterus index □ Takata ÷ ■ Takata + ■ Takata ++ ■ Takata +++
 ▨ Albumin □ Globulin
 (See case record of patient No. 7.)

in hospital for miscarriage; when discharged from the Surgical Department was sub-febrile with nausea. Three weeks later yellow. When admitted here she had thus been ill for about four months. On admission the liver was not found to be enlarged. She complained of tiredness and nausea. Thereafter the jaundice increased. About 3 months after admission there was ascites. Diuresis was high, however (she received diuretica), and during the next months the ascites disappeared. The jaundice left her and she was discharged as well. Examined 5 months later she was in good health.

On admission the serum albumin was normal; it fell during the following months corresponding to the appearance of ascites, whereafter it rose as the ascites subsided. The globulin fluctuated in the opposite directions. Takata was positive in hospital, negative at the later examination. On admission the galactose test was positive (5.2 g). During the first 14 weeks in hospital the temperature was sub-febrile.

Case Book 1154/45. 10-4 to 1-9-45.

Patient No. 7, case record (figure III).

Female, 66 years. Menopause at 50th year. Four weeks before hospitalization tiredness, loss of appetite and feverish. Three weeks before hosp. jaundice. While in hosp. the icterus index fell and the condition improved. During most of the hosp. period temperature

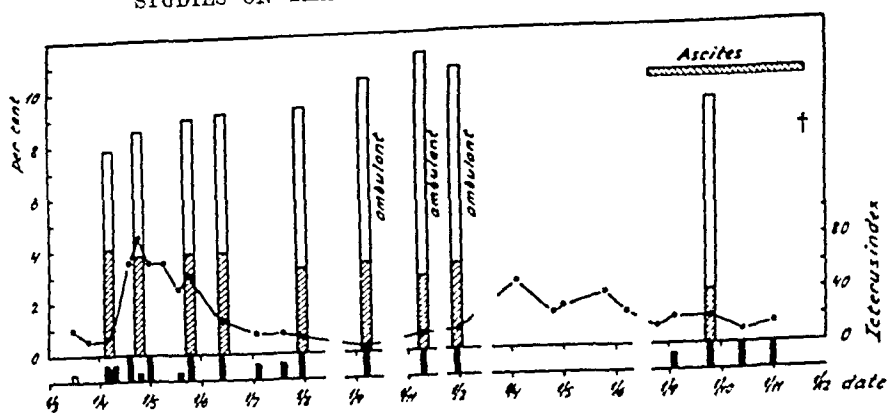


Figure IV

Woman 55 years old. Duration of illness before admittance: 2 weeks.

— Icterus index □ Takata ÷ ■ Takata + ■ Takata ++ ■ Takata +++
 ▨ Albumin □ Globulin
 (See case record of patient No. 11.)

sub-febrile. Liver biopsy a month after admission showed Hepatitis subchronica mag. gr.

Discharged after about 5 months. After-examination 2 months later: patient tired, no appetite. No jaundice. No ascites.

Takata positive throughout observation period. Albumin percentage normal, globulin high.

When examined 2 years after discharge she stated she had felt well for about a year. Takata and icterus index now normal. Serum proteins likewise.

Case Book 1055/45. 13-3 to 9-8-45.

1596/46. 7-4 to 18-11-46.

Patient No. 11, case record (Figure IV).

Female, 55 years. Menopause when 50. Previously well. Fourteen days before hospitalization nausea. Two days later jaundice, and simultaneously pyrexia (38.5). On admission the liver was not enlarged. A tumour was found, corresponding to the uterus. Galactose test negative (1.9 g). While in hosp. temperature sub-febrile, occasionally round about 39°. Cystitis in periods, with tenesmia and pyuria. She complained of nausea, tiredness and slight pruritus. In the first hosp. period her weight fell from 63 to 54 kg. The jaundice decreased and the general condition improved somewhat. After discharge she still had nausea and tiredness. Admitted again 8 months after discharge. Ascites observed 3½ months later. Died 7 months after 2nd admission in Coma hepaticum. Post mortem: Subacute atrophy of the liver and Fibroma uteri.

The serum proteins, which were followed from about a month after the onset of the disease, showed high globulin percentage and low al-

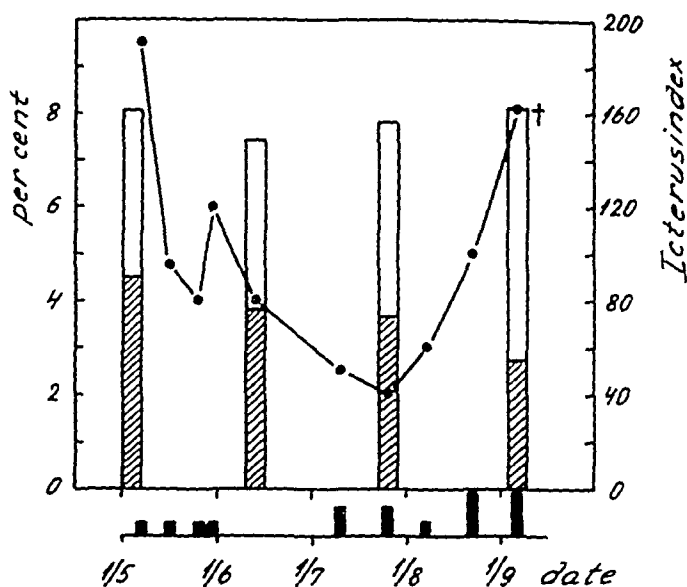


Figure V

Woman 65 years old. Duration of illness before admittance: 4 weeks.

— Icterus index □ Takata ÷ ■ Takata + ■ Takata ++ ■ Takata +++
 ▨ Albumin □ Globulin
 (See case record of patient No. 18.)

bumin. Takata, which was negative at the first examination, became positive and continued positive later.

Case Book 1207/45. 4-5 to 11-9-45.

Patient No. 18, case record (Figure V).

Female, 65 years. Menopause when 50. For many years had attacks of cholelithiasis, the last one 20 years before hospitalization. Fifteen years ago *Fractura colli femoris sin.* Thirteen years ago *Pleuritis dx.* During past four weeks steadily increasing jaundice, and simultaneously slight epigastric pains, nausea and tiredness.

On admission the liver felt just below the curvature, rather firm. An ovarian cyst, the size of a man's head, found on the right side. Galactose test doubtfully positive (2.9 g). Patient complained of nausea, pruritus, and occasionally of abdominal pains. There was transitory dysuria, accompanied by pyuria and coliuria. Jaundice again increased after 3 months in hosp. She complained of tension in the abdomen. Hepatargia set in, during which a single black-coloured vomition. Death occurred about 4 months after admission. Temperature normal most of the time, occasionally sub-febrile.

Post mortem: Sub-acute atrophy of the liver and unilocular ovarian cyst.

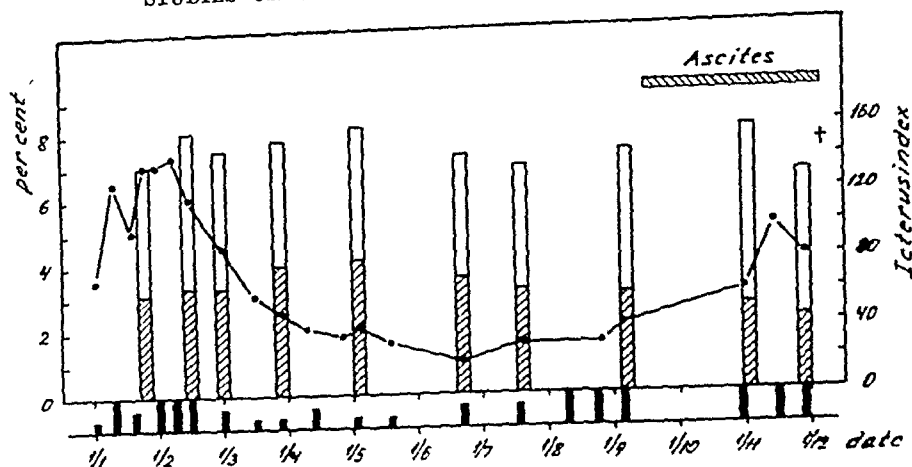


Figure VI

Woman 73 years old. Duration of illness before admittance: abt. 6 months.

— Icterus index □ Takata - ■ Takata + ■ Takata ++ ■ Takata +++
 ▨ Albumin □ Globulin
 (See case record of patient No. 20.)

The albumin percentage fell and the globulin rose while in hosp. and simultaneously the Takata reaction increased in strength.

Case Book 1178/45. Hosp. 31-12-44 to 5-9-45.

25-10-45, died 1-12-45.

Patient No. 20, case record (Figure VI).

Female, 73 years. Hosp. 23 years previously for hysterectomy. During past six months tired with loss of weight. Urine stated to have been dark in periods. Three weeks before admission jaundice observed. On admission liver found to be much enlarged, reaching to Crista iliaca. At first a liver or kidney tumour was suspected. Liver biopsy revealed widespread acute hepatitis. The galactose test was positive (6.7 g). While in hosp. patient complained of tiredness and vertigo as well as pain in the *right* side of the chest. The liver slowly decreased in size. The jaundice subsided and the general condition improved somewhat, wherefore she was sent home after 8 months in hosp. Two weeks later she had ascites and oedema in the loins. Admitted six weeks after discharge. Now complained of pain in the epigastrium and tension over the whole abdomen. Was very drowsy. Died in hepatic coma 5 weeks after second admission. Post mortem revealed sub-acute liver atrophy. The serum proteins had been followed for a year. During the first months the albumin percentage increased, and thereafter fell slowly. The serum globulin percentage varied counter to the albumin percentage. Correspondingly, the Takata reaction was strongest at the beginning and the end of the observation period. The temperature was normal during both periods in hospital.

Summary.

The author presents case reports and diagrams of variations in the icterus index, Takata reaction, serum albumin and serum globulin percentages for 18 women and 3 men with severe hepatitis. The patients were all followed up to their death or recovery. The serum proteins vary, the albumin falling when the globulin rises, and vice versa. It is confirmed that the variations in the albumin follow the clinical course of the disease. No patient survived a fall in the albumin concentration below 2.48 %. During the attack six patients who recovered (Nos. 1—6), had ascites and oedema which disappeared. The serum albumin fell during the ascites period, rising again when the ascites disappeared.

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Studies on Sahli's High Pressure Stasis.

By

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In 1901 Hermann Sahli (1) reported that the systolic blood pressure often rose 10—30 mm mercury in cardiac insufficiency with signs of stasis, and that it fell when the stasis was abolished (high pressure stasis, »Hochdruckstauung«).

Subsequently several authors have dealt with this phenomenon. Most of them — *e. g.* Lang and Manswetowa (2), Loschkarewa (3), Meyer and Mullen (5), Fishberg (6, 7), Hammerström (8) — have taken it to be a really characteristic feature, whereas Frehse (4) was unable to subscribe to this view. The syndrome was taken to be elicited by anoxemia or hypercarbonemia accompanied by peripheral vasoconstriction. Most works on this subject, however, are in want of a satisfactory control material. For, as is well known, the blood pressure falls on confinement to bed, and hence it is not warrantable without a control material to draw any conclusion as to whether a fall in blood pressure is due to abolition of the stasis or to the bed-rest *per se*.

In order to look into this question, therefore, in the Medical Department B of the Rigshospital, Copenhagen, I have collected a material comprising 224 patients with cardiac disease and 50 patients without any heart lesion or any other disease influencing the blood pressure in a degree worth mention. Of the 224 heart patients 103 showed definite signs of stasis as clinical and roentgenological congestion of the lungs, ascites, congestion of the liver or considerable edema of the lower extremities. Comparative

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studies have been made on the course of the systolic and diastolic blood pressure from the day of admission to the discharge in three groups of patients: 1) patients with decompensated cardiac disease and symptoms of stasis who improved, 2) heart patients without symptoms of stasis, and 3) patients without any heart lesion. If Sahli's high pressure stasis be a reality, it will be reasonable to expect that in the patients with symptoms of stasis the systolic pressure will prove to fall more frequently and more markedly on abolition or marked improvement of the stasis than in the remaining two groups.

Of the 103 patients with stasis 76 improved considerably. Among these patients 12 were suffering from a mitral lesion with stasis in the lungs alone. But, as the significance of pulmonary stasis in mitral affection differs from that of stasis elsewhere, being required for the compensation (9), these patients are here left out of account. In this group, then, there remain 64 decompensated heart patients who improved.

For comparison we have a group of heart patients without stasis — altogether 121 — of whom 71 improved, while the state of the remaining 50 was unchanged at their discharge. The latter patients are not included in this account.

The third group comprises 50 patients without any heart lesion or any other disease influencing the blood pressure noticeably.

The frequency of a fall in the systolic and diastolic pressures observed in the various groups is recorded in Tables 1 and 2. The magnitude of the falls varies between 10 and 50 mm mercury, falls under 10 mm being reckoned as unchanged blood pressure. All the measurements were made indirectly *ad modum* Riva-Rocci.

From Tables 1 and 2 it will be noticed that a fall in the systolic as well as the diastolic pressure is not more frequent among the decompensated heart patients with subsiding stasis than among heart patients with stasis. Among the patients without any heart lesion the fall in diastolic pressure appears to be less pronounced but presumably this is due to the circumstance that the average age of these patients is somewhat lower than that of the two other groups, making the blood pressure generally somewhat lower, on which account presumably a fall in diastolic pressure will be less frequent (8).

I then looked into the distribution of the falls in blood pressure on the three groups in the patients whose systolic

Table 1.

Frequency of Variations in the Systolic Blood Pressure in the Different Groups.

	No. of patients	Fall of 10—50 mm	Rise of 10—50 mm	Pressure unchanged
Decompensated heart patients with signs of stasis	64	37 (57.8 %)	19 (29.7 %)	8 (12.5 %)
Heart patients without stasis	71	43 (60.6 %)	15 (21.1 %)	13 (18.3 %)
Patients without cardiac disease	50	30 (60.0 %)	10 (20.0 %)	10 (20.0 %)

Table 2.

Frequency of Variations in the Diastolic Blood Pressure in the Different Groups.

	No. of patients	Fall of 10—50 mm	Rise of 10—50 mm	Pressure unchanged
Decompensated heart patients with signs of stasis	64	33 (51.6 %)	17 (26.6 %)	14 (21.9 %)
Heart patients without stasis	71	41 (57.7 %)	17 (23.9 %)	13 (18.3 %)
Patients without cardiac disease	50	21 (42.0 %)	19 (38.0 %)	10 (20.0 %)

pressure at some juncture of their stay in the hospital was under 150 mm, and who did not have coronary thrombosis. For in patients with definite hypertension — *i. e.*, patients whose systolic pressure throughout the hospitalization is over 150 mm — the blood pressure will fall more frequently and more markedly than in other patients (8). Thus a skew distribution of these patients on the groups will give an inaccurate impression. Here it is to be mentioned that there is no definite difference in the magnitude and frequency of the fall in blood pressure between the decompensated and compensated cases of hypertension. Here the cases of coronary thrombosis are left out of consideration too because at the acute stage of this condition the blood pressure most often is lower than usual and then rises after the acute stage (9). So an unequal distribution of such patients on the three groups may likewise give a false impression.

Classifying the present material in this way, we have one group of 50 patients with decompensated cardiac disease, presenting signs of stasis that improve, one group of 42 compensated heart patients without stasis, also improving, and one group of patients without any heart lesion. The frequency of fall in the blood pressure encountered in the various groups is evident from Tables 3 and 4.

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Table 3.

Frequency of Variations in the Systolic Pressure in the Different Groups (Coronary Thrombosis and Hypertension not Included).

	No. of patients	Fall of 10—50 mm	Rise of 10—50 mm	Pressure unchanged
Decompensated heart patients without stasis ..	50	29 (58.0 %)	15 (30.0 %)	6 (12.0 %)
Heart patients without stasis	42	28 (66.7 %)	10 (23.8 %)	4 (9.5 %)
Patients without heart lesion	50	30 (60.0 %)	10 (20.0 %)	10 (20.0 %)

Table 4.

Frequency of Variations in the Diastolic Pressure in the Different Groups (Coronary Thrombosis and Hypertension not Included).

	No. of patients	Fall of 10—50 mm	Rise of 10—50 mm	Pressure unchanged
Decompensated heart patients with stasis	50	28 (56.0 %)	13 (26.0 %)	9 (18.0 %)
Heart patients without stasis	42	6 (61.9 %)	11 (26.2 %)	5 (11.9 %)
Patients without heart lesion	50	21 (42.0 %)	19 (38.0 %)	10 (20.0 %)

As will be noticed, this makes no decisive difference in the results.

Then, in order to see whether the blood pressures fall to the same extent in the three last-mentioned groups of patients, both the systolic and the diastolic pressure have been followed for each patient from week to week. This was practicable in part as far as the first weeks in hospital are concerned, as in this period the blood pressure was measured about once a week. Later in the stay, unfortunately, this was done with the same regularity and frequency. Then the mean values are calculated for the systolic and diastolic pressures within each group on admission, after 1 week, after 2 weeks, after 3 and 4 weeks. Within this period of 4 weeks, as a rule, the patients admitted with cardiac insufficiency will have improved considerably. The calculated values are recorded in Tables 5 and 6.

The values recorded in Tables 5 and 6 are presented graphically in Figs. 1 and 2.

It will be noticed that several measurements are wanting for the third and fourth weeks, so that it hardly is quite correct to compare the various mean values, as the numbers of patients from which these values are calculated are far from identical.

Table 5.

Fall in Systolic Pressure in the Different Groups.

a. Group of decompensated heart patients (average age: 54.8 years).					
On admission	134	mm Hg, calculated from 50 cases			
After 1 week	126	" " " " "	47	"	
" 2 weeks	122	" " " " "	47	"	
" 3 "	121	" " " " "	32	"	
" 4 "	122	" " " " "	23	"	
b. Group of heart patients without stasis (average age: 58.6 years).					
On admission	137	mm Hg, calculated from 42 cases			
After 1 week	124	" " " " "	42	"	
" 2 weeks	122	" " " " "	35	"	
" 3 "	129	" " " " "	27	"	
" 4 "	122	" " " " "	16	"	
c. Group of patients without heart lesion (average age: 42.1 years).					
On admission	124	mm Hg, calculated from 50 cases			
After 1 week	115	" " " " "	32	"	
" 2 weeks	113	" " " " "	34	"	
" 3 "	107	" " " " "	14	"	

Table 6.

Fall in Diastolic Pressure in the Different Groups.

a. Group of decompensated heart patients (average age: 54.8 years).					
On admission	76	mm Hg, calculated from 50 cases			
After 1 week	71	" " " " "	47	"	
" 2 weeks	69	" " " " "	47	"	
" 3 "	67	" " " " "	32	"	
" 4 "	68	" " " " "	23	"	
b. Group of heart patients without stasis (average age: 58.6 years).					
On admission	79	mm Hg, calculated from 42 cases			
After 1 week	71	" " " " "	42	"	
" 2 weeks	66	" " " " "	35	"	
" 3 "	76	" " " " "	27	"	
" 4 "	69	" " " " "	16	"	
c. Group of patients without heart lesion (average age: 42.1 years).					
On admission	64	mm Hg, calculated from 50 cases			
After 1 week	60	" " " " "	32	"	
" 2 weeks	64	" " " " "	43	"	
" 3 "	60	" " " " "	14	"	

But, as it is an altogether accidental matter which patients are missing, the values still give a very good idea about the fall of the blood pressures.

Both the systolic and diastolic curves are seen not to differ much mutually. If Sahli's high pressure stasis were a really characteristic phenomenon, we would expect the fall of the curves to be greater for the patients with cardiac insufficiency.

It will also be noticed that the height of the blood pressures on admission to the hospital is practically the same for the two groups of heart patients (average age, 54.8 and 58.6 years respectively). This, too, goes against Sahli's high pressure stasis, as otherwise

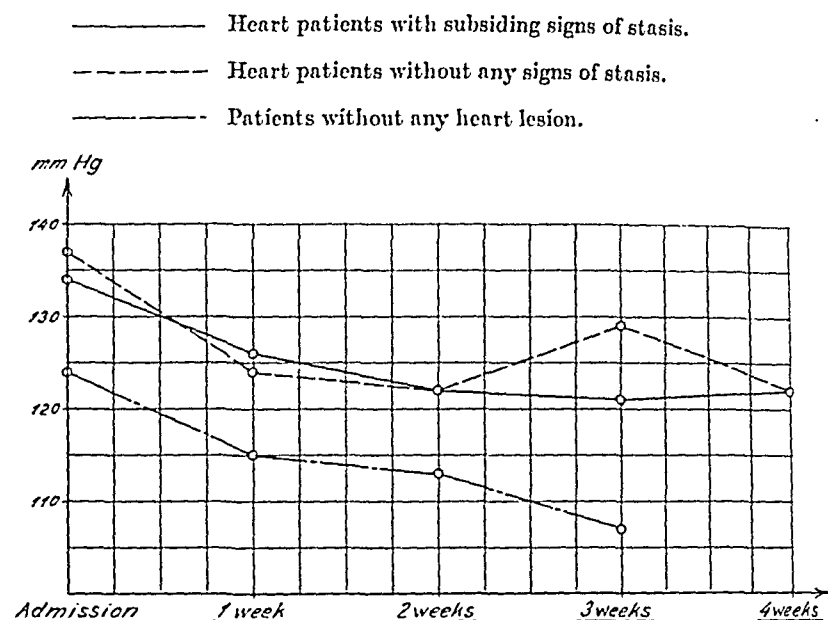


Fig. 1. Course of the systolic pressure during hospitalization.

we would expect the blood pressure to lie at a somewhat higher level in the patients with cardiac insufficiency. The somewhat lower level of the blood pressures in the patients without any heart lesion is quite in keeping with their lower average age (42.1 years).

On reckoning the blood pressure fall in each patient as the difference between the pressure on admission and at the discharge, and calculating the average fall for each group of patients, we get the following values:

Fall in systolic pressure in

50 heart patients with signs of stasis: 28 mm Hg

42 " " without " " " 28 " "

50 patients without any heart lesion: 21 " "

Fall in diastolic pressure in

50 heart patients with signs of stasis 21 mm Hg

42 " " without " " " 19 " "

50 patients without any heart lesion: 17 " "

Here, too, we find no decisive difference between the group with decompensated heart lesion and the two other groups.

Considering the individual patients with a large fall in systolic pressure, a fall over 30 mm was observed in 11 of the 50 patients

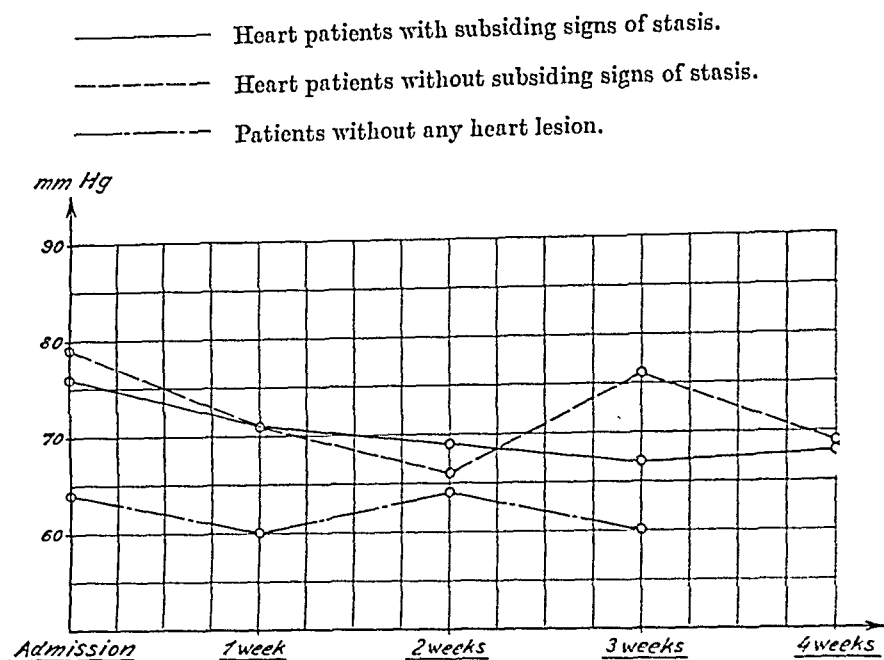


Fig. 2. Course of the diastolic pressure during hospitalization.

with decompensated heart lesion, in 9 out of the 42 patients with well-compensated heart lesion, and in 6 of the 50 patients without any heart lesion. Thus, in this respect, there is no significant difference between the first two groups. Presumably the lower figure for the last group is attributable to the lower average age of these patients, making individual large falls in blood pressure less frequent (8).

The total outcome of this account will then be that falls in the systolic and diastolic blood pressures are not more frequent and larger in improving heart patients with subsiding signs of stasis than in other hospitalized patients. The falls in blood pressure observed in these patients, which previously have made clinicians reckon with Sahli's high pressure stasis as a reality, merely signify that the blood pressure often falls when such patients are hospitalized and kept at rest.

Summary.

The view advanced by Sahli in 1901 — that stasis causes a rise in the arterial blood pressure — is here made the subject of control examination.

No difference is found in the fall of the blood pressure during hospitalization in decompensated heart patients with improvement of their stasis symptoms, in well-compensated heart patients and in patients without cardiac disease.

Thus Sahli's high pressure stasis cannot be looked upon as a reality.

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Evaluation of Dubos' Medium in Routine Diagnostic Examination of Tubercle Bacilli from Pathologic Material.

By

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(Submitted for publication January 28, 1948.)

In 1945, Dubos (1) reported that water soluble lipids, phospholipids and polyoxyalkylene derivatives of oleic or stearic acid, for instance, stimulated the multiplication of *Mycobacteria* and permitted a submerged growth in synthetic liquid media. For initiation of growth by small inocula addition of serum albumin was required. In subsequent papers Dubos and his collaborators (2, 3, 4) analyzed in detail the factors affecting the growth of tubercle bacilli in fluid media, in particular the effect of water soluble lipids and of serum albumin.

These reports suggested the possibility that such media would be of great diagnostic value in the examination of pathologic material, and Foley's (5) small series, 29 positive specimens, seems to confirm this suggestion.

In order to evaluate the diagnostic value of Dubos' medium in routine work the following study was made. Since January 1947 all specimens received for routine examination of tubercle bacilli were examined by culture both with this and with Löwenstein's medium as well as by guinea pig inoculation. The results obtained with these three methods have then been compared.

Materials and Methods.

Pleural effusions, gastric aspirations, spinal fluids and urine specimens were centrifuged and the sediment diluted to 5 ml. Before inoculation, 5 ml of the different specimens (except spinal fluid) were

then digested with an equal volume of 6 per cent sulphuric acid for 10 minutes at room temperature, then neutralized to approximately pH 7.0 with 4 per cent sodium hydroxide. The digest was centrifuged and the sediment diluted with 4 ml of physiologic saline solution. Two ml of this digested, concentrated specimen was then injected into the guinea pig. After six weeks the animals were killed and examined according to the methods in general use. Each of two bottles containing Dubos' medium were inoculated with 0.5 ml of the treated specimen and each of five test tubes containing Löwenstein's medium with 0.2 ml. Thus Dubos' and Löwenstein's media were respectively inoculated with one ml of the specimen, *i. e.* half the inoculum used in the guinea pigs.

In this study Dubos' medium has had the following composition:

Basal medium.

Casein hydrolysate ¹	1.0 gm
Na ₂ HPO ₄ · 12H ₂ O	6.3 gm
KH ₂ PO ₂	1.0 gm
Na ₃ citrate · 2H ₂ O	1.8 gm
MgSO ₄ · 7H ₂ O	0.6 gm
Water	ad 1000 ml

The pH is adjusted to 7.0—7.2.

This basal medium is then autoclaved for 1 hour at 120° C.

After autoclaving Tween 80 (polyoxethylene sorbitan monooleate), glucose and serum albumin are added aseptically. The final concentration of Tween 80 was 0.05 per cent, of serum albumin and of glucose 0.5 per cent. For further details see Dubos and Davis (2). Dubos (6) has later changed the composition of the basal medium and included other minerals such as Fe, Ca, Zn and Cu. This change in the basal medium in our hands did not improve the growth, and we have therefore only used the original composition in this study.²

The medium is then distributed in 5 ml amounts in metal-capped small bottles and then inoculated.

The inoculated media, both Löwenstein and Dubos, were incubated at 37° C and examined every third day during three weeks for evidence of growth. After that time examination was made once a week for another three weeks. On each occasion the Dubos cultures were smeared and stained according to Ziehl-Neelson. If visible growth had occurred smears were made from a loop; if not, 0.5 ml of the culture was centrifuged and the sediment was smeared.

In all specimens positive only by culture, the presence of virulent tubercle bacilli was checked by a subsequent guinea pig test.

¹ We have used aminosol Vitrum, an enzymatic digest of casein.

² As Davis and Dubos (4) have shown, Tween 80 can be replaced oleic acid. In 59 positive cultures the same volume of the treated specimens were simultaneously inoculated both on a medium containing Tween 80 and on a medium containing oleic acid. No significant difference could be demonstrated between these two media in respect of the number of positive cultures and in the rapidity with which the cultures grow out.

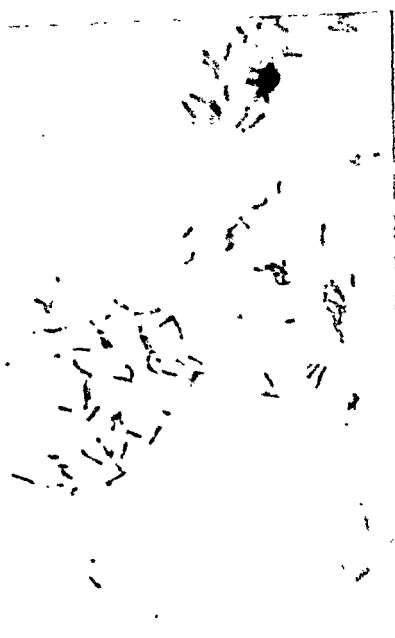


Fig. 1 a.

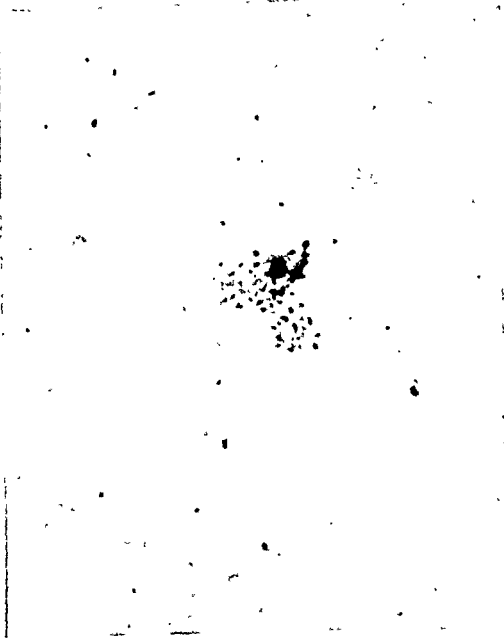


Fig. 1 b.

Results.

With the aid of Dubos' medium it was possible to obtain a rapid and diffuse growth of tubercle bacilli. The growth rate depended to a considerable extent on the size of the initial inoculum. With large inocula growth sometimes was visible within 72 hours, with very small inocula two or three weeks elapsed before any growth could be demonstrated. These rapidly multiplying, young cultures often showed characteristic morphologic features. The bacilli were often slightly thicker and clumsy, many times showed a beaded or banded appearance and were often not acid-fast (fig. 1 a, b). As a rule the bacilli occurred in a non- or in an incomplete acid-fast form when a positive growth could be demonstrated for the first time. In older cultures the acid-fastness was more marked or fully developed. The young cultures could also not resist digestion with acids or alkalis. On the other hand, when tubercle bacilli were grown on the surface of a solid medium, prepared by adding 1.5 per cent agar to the same nutrient solution as that used in the liquid medium, acid-fastness was more regularly apparent when the first growth could be detected. The cause of this difference will be investigated. With further cultivation in the liquid



Fig. 2.

medium no changes in the characteristic morphology, staining properties and virulence of tubercle bacilli could be demonstrated. In the stained preparations they often occurred in a characteristic tail-shaped arrangement (fig. 2).

In all, 1,379 specimens have been examined and of these 436 became positive by culture or by guinea pig inoculation or by both of these methods (table 1). About one fourth of the positive specimens examined were obtained from patients with proven tuberculosis, the rest were obtained from cases in which tuberculosis was suspected clinically or had to be ruled out. Table 1 also shows the composition of the pathologic material and the number of positive tests obtained with the three different procedures. In 25 instances where cultures were positive the animals died from intercurrent disease before the test was terminated. The presence of virulent tubercle bacilli was confirmed by guinea pig inoculation from the positive cultures. If these 25 instances are excluded the total number of positive specimens where all three procedures could be carried out is thus 411.

About 50 per cent (193 cases) were simultaneously positive by culture on Löwenstein's or on Dubos' media and by guinea pig inoculation. In 8 instances positive results were obtained only with

Table 1.

Composition of Material and Number of Specimens Positive by Means of Löwenstein and Dubos Culture and Guinea Pig Inoculation.

Löwenstein culture	neg.	pos.	pos.	pos.	pos.	neg.	neg.	neg.	pos.	pos.	neg.	Total number positive specimens
Dubos culture	neg.	pos.	pos.	neg.	neg.	pos.	neg.	pos.	pos.	neg.	pos.	
Guinea pig	neg.	pos.	neg.	neg.	pos.	pos.	pos.	neg.	— ¹	— ¹	— ¹	
Material examined	No specimens											
Sputum	107	65	1	1	7	8	11	4	3	1	1	102
Gastric aspiration	530	64	5	6	30	12	64	13	7	2	3	206
Pleural effusion..	138	29	0	1	12	4	9	4	4	0	0	63
Urine	92	22	0	0	7	2	3	3	2	0	1	40
Spinal fluid	20	4	0	0	0	0	3	0	1	0	0	8
Miscellaneous including Joint fluid	56	9	0	0	3	3	1	1	0	0	0	17
Pus												
Autopsy material												
Total	943	193	6	8	59	29	91	25	17	3	5	436

Löwenstein's culture, in 25 cases only with Dubos' culture and in 91 cases only by guinea pig test.

In table 2 the percentage of positive tests obtained with the three different procedures are given. Of all positive specimens 91 per cent were positive by guinea pig test and 78 per cent by culture. Culture on Löwenstein's and on Dubos' media yielded about the same percentage of positive tests. The difference in positive tests between guinea pig inoculation and cultivation depends to a large extent upon the composition of our material. Of the positive specimens about 50 per cent (194 cases) were obtained from gastric aspirations. In this group 33 per cent (64 cases) were positive only by guinea pig test. If this group of specimens is excluded the rest of the material showed on the whole as many positive tests by culture as by guinea pig inoculation. The reason why guinea pig tests gave 33 per cent more positive tests than culture in the group of gastric aspirations to some extent depends upon the experimen-

¹ The guinea pigs died from intercurrent disease during the test.

Table 2.

Percentage of Positive Tests by Culture and by Guinea Pig Inoculation for Different Kinds of Pathologic Material.

Material cultured	No positive specimens	Per cent positive by guinea pigs	Per cent positive by Löwenstein's or Dubos' culture	Per cent positive by Löwenstein's culture	Per cent positive by Dubos' culture
Gastric aspiration	194	87.6	67.0	54.1	48.4
Sputum	97	93.1	88.6	76.3	80.4
Pleural effusion	59	91.5	84.8	71.2	62.7
Urine	37	91.9	91.9	78.4	73.0
Miscellaneous ..	17	94.1	94.1	70.6	88.2
Spinal fluid ..	7	100.0	57.2	57.2	57.2
Total	411	90.5	77.9	64.7	61.6

Table 3.

Incubation Time in Days when Positive Culture was Obtained.

Material cultured	No. specimens	Dubos' medium								Löwenstein's medium							
		0	6-9	10-14	15-21	22-28	29-35	36-42		10-14	15-21	22-28	29-35	36-42			
Sputum	65	18	18	11	14	4	0	0	0	21	31	7	3	3			
Gastric aspiration ..	89	6	8	8	32	19	13	3	7	27	28	16	11				
Pleural effusion....	25	2	6	5	8	2	1	1	6	8	7	1	3				
Urine	27	—	—	6	7	7	5	2	—	12	11	4	—				
Miscellaneous	11	—	1	5	3	1	1	—	1	3	4	2	1				
Total	217	26	33	35	64	33	20	6	35	81	57	26	18				

tal procedure we used. Dubos' and Löwenstein's media were inoculated with only one half of the inoculum used for guinea pig inoculation. Gastric aspirations usually contain small numbers of tubercle bacilli and the chances to obtain positive results must therefore be smaller when only half the inoculum is used.

Table 2.

Percentage of Positive Tests by Culture and by Guinea Pig Inoculation for Different Kinds of Pathologic Material.

Material cultured	No positive specimens	Per cent positive by guinea pigs	Per cent positive by Löwenstein's or Dubos' culture	Per cent positive by Löwenstein's culture	Per cent positive by Dubos' culture
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Urine	37	91.9	91.9	78.4	73.0
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Sputum	65	18	18	11	14	4	0	0	0	21	31	7	3	3			
Gastric aspiration ..	89	6	8	8	32	19	13	3	7	27	28	16	11				
Pleural effusion	25	2	6	5	8	2	1	1	6	8	7	1	3				
Urine	27	—	—	6	7	7	5	2	—	12	11	4	—				
Miscellaneous	11	—	1	5	3	1	1	—	1	3	4	2	1				
Total	217	26	33	35	64	33	20	6	35	81	57	26	18				

tal procedure we used. Dubos' and Löwenstein's media were inoculated with only one half of the inoculum used for guinea pig inoculation. Gastric aspirations usually contain small numbers of tubercle bacilli and the chances to obtain positive results must therefore be smaller when only half the inoculum is used.

The time of incubation when a positive culture was obtained is given in table 3. In 217 specimens the culture was positive with both Dubos' and Löwenstein's media. With Dubos' medium, in 12 per cent (26 cases) the culture was positive before six days, in 25 per cent (59 cases) before ten days, and in 44 per cent within fourteen days. Before ten days no culture was positive with Löwenstein's medium and within 14 days only 16 per cent.

Discussion.

From these results it is evident that Dubos' medium is of the greatest value in routine diagnostic examination of tubercle bacilli from pathologic material. Because of our experimental procedure the results obtained by culture and by guinea pig inoculation are not wholly comparable. The inoculum used for the guinea pigs was twice the inoculum used for the cultures, and therefore the chances for a positive test must be greater by guinea pig inoculation than by culture. In some kinds of specimens, such as gastric aspirations where the number of bacilli usually are small, this discrepancy was more marked. If this group is excluded guinea pig inoculation and culture gave about the same percentage of positive tests, 93 and 88 per cent respectively. There was no difference in the number of positive tests obtained by Löwenstein's and Dubos' medium. The great advantage of Dubos' medium is the rapidity with which it is possible to obtain positive results in many cases. Of the specimens where both Dubos' and Löwenstein's cultures were positive 25 per cent were positive on Dubos' medium within nine days and no specimen on Löwenstein's medium. The corresponding figures for fourteen days incubation were 44 per cent and 16 per cent respectively.

In spite of the many advantages of Dubos' medium, this liquid medium is not absolutely ideal for routine diagnostic examination of tubercle bacilli from pathologic material. One of the most important reasons for this is that the cultures often are contaminated with other bacteria. Because of the possibility that the culture is contaminated it is, from ocular examination, impossible to judge whether growth of tubercle bacilli is present or not. On each occasion smears must be made and even in stained preparations it is often difficult to demonstrate tubercle bacilli especially when the cultures are contaminated. In many cases a diligent search of the smears was required before a few small clumps of

Table 4.

Comparison of the Incubation Time Required to Obtain Positive Culture on Solid and on Liquid Dubos' Medium.

Incubation in days when culture became positive.

No growth	Solid		Liquid		Total number	
	Sol.	Liq.	Sol.	Liq.	Sol.	Liq.
> 6	6—9	10—14	15—21	22—28	29—35	36—42
2	—	—	—	—	—	—
7	4	5	27	4	1	—
83	—	—	—	—	—	—

tubercle bacilli could be demonstrated. These repeated examinations are very time-consuming and increase the risk for contamination. It is also possible that the growth of contaminant bacteria can suppress the growth of tubercle bacilli. Trials with sulfa-drugs and penicillin in order to prevent the growth of contaminant bacteria have been discouraging; in the concentrations required these substances inhibited the growth of tubercle bacilli.

In order to diminish the disturbing influence of contamination and to facilitate the demonstration of tubercle bacilli, we therefore tried the use of a solid medium prepared by adding 1.5 per cent agar to the fluid medium. The question as to whether tubercle bacilli obtained from pathologic material grew with the same rapidity and yielded the same number of positive cultures on solid as on liquid media was examined in the following way. Autopsy material from 83 different and positive guinea pig tests was used. This kind of material was chosen because it gives a high percentage of positive cultures without contamination. The same volume of the treated specimens was inoculated on both solid and liquid Dubos' media. The results are summarized in table 4. Except in two cases positive cultures were obtained both on solid and liquid media, *i. e.* in 98 per cent. On the solid medium growth could be detected earlier than on the liquid medium, within nine days in 72 cases the culture was positive on solid medium against 49 on liquid medium.

The solid medium thus at least gives the same number of positive tests as the liquid medium, and on the solid medium in 30 per cent of the cases growth was evident earlier than on the liquid medium. The solid medium also offers other advantages. It makes the demonstration of tubercle bacilli easier especially when the cultures are contaminated. The evaluation of the solid medium in routine examination for tubercle bacilli is in progress. Prelim-

inary results indicate that it will be of greatest value in the laboratory diagnosis of tuberculosis.

Further improvements are still possible. If contamination by other bacteria could be eliminated and if the small colonies of tubercle bacilli could be made more easily detectable, by means of a dye in the medium, for instance, it would mean a progress and a simplification of this technic in routine diagnostic work. We have found that the addition to the medium of some vital dyes, especially basic triphenyl methane dyes which are known to have affinity for the tubercle bacillus, colors the colonies and to some extent inhibits the growth of contaminant bacteria without suppressing the growth of tubercle bacilli. Further studies to find the most suitable dye and its optimal concentration in the medium are in progress.

Summary.

Dubos' fluid medium has been used in the routine diagnostic examination of 1,379 specimens obtained from various pathologic material. Simultaneously the same specimens have been examined by cultures on Löwenstein's medium and by guinea pig inoculation. Four hundred and thirty-six of the specimens were positive. The results with three methods have then been compared.

Dubos' liquid medium is of great value for the laboratory diagnosis of tuberculosis. It is not, however, ideal for routine examination of pathologic material. A solid medium containing the same nutrient solution offers greater advantages. The possibilities for further improvement of the medium are discussed.

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Book Review.

HANS SELYE: *Textbook of Endocrinology*. 914 p. with numerous figures. Edited 1947 by Acta Endocrinologica, Université de Montréal, Montréal, Canada. Price: \$ 10.24.

The author is M. D., Ph. D. (Prague), D. Sc. (McGill), F. R. S. (Canada), Professor and Director of the Institut de Médecine et de Chirurgie expérimentales, Université de Montréal.

I think the best way of reviewing this important book is to cite a part of the preface, written by Professor Bernardo A. Housay, Buenos Aires, Nobel price winner 1947. He writes:

»This book represents a critical and concise, orderly presentation of what is most important in the immense collection of facts of modern endocrinology, a science so vast that there are specialists in numerous branch problems and few who know this entire field of study. The author of this book not only succeeded in describing the accumulated facts but also to crystallize those fundamental principles which are derived from them, thus emphasizing that there is only a science of the general and that from time to time the need for a great synthesis arises.

In such a vast field of knowledge it is impossible that a single person could dominate all aspects of endocrinology with equal competence. — Selye possesses exceptional and probably unique conditions and abilities for this. He owns the largest endocrinologic library in the world, a collection which is admirably organized. His exceptional command of numerous languages allows him to understand the characteristic thoughts and cultures of diverse countries and to avoid the provincialism so common even in the greatest nations. Furthermore, in order to make the book an objective exposition of contemporary endocrinology, the author has submitted each chapter for revision to some of the most eminent authorities in that particular field. Selye is a brilliant teacher and knows the art of how to explain things clearly and

methodically. He possesses a personal knowledge of the major part of experimental endocrinology, in its anatomic and physiologic aspects; furthermore, he has contributed important original studies, executed with skilful technique, to the development of the science. His exceptional erudition can be appreciated in this book which is a basic and unitarian presentation of the problem. The limitation of space obliges him sometimes to be somewhat dogmatic in his exposition or to condense the information; however, the latter may be expanded by reference to the bibliography recommended by the author.

In addition to being a text, the book is also an atlas since it contains illustrations of everything that can be photographed (histology, crystals of hormones, experiments, X-rays of clinical cases).

This book, in spite of its exceptional qualities, does not oblige to a dogmatic adherence since it describes an unfinished field of knowledge. Reading of the book will be an indispensable point of departure for beginners as well as for specialists; the former will subsequently complete their knowledge when necessary by the study of clinical and experimental material and of more detailed treatises.

It is indubitable that this book will possess an historic importance, since it is the most complete synthesis of endocrinologic facts published up to date and it will disseminate these with unequalled efficiency. It will furthermore stimulate studies and promote investigations which will help the progress of endocrinology. Like all texts it will not satisfy all specialists and critics in every detail; however it is indubitable that this book will have a decisive influence upon the dissemination of exact data and the promotion of investigations conducive to the progress of endocrinology.» So does Houssay express his opinion of this book.

I. Holmgren.

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From the Pharmacological Dept. of Karolinska Institutet and the Endocrine Division of the Medical Clinic of Serafimerlasarettet, Stockholm.

A Comparison of Oral and Intravenous Dextrose Tolerance Tests in Healthy Subjects.

By

LEONARD GOLDBERG and ROLF LUFF.

(Submitted for publication December 19, 1947.)

Hofmeister (1889) introduced the conception of »glucose tolerance» denoting the amount of glucose which develops glycosuria. »Glucose tolerance» has also been used to indicate the degree and duration of the hyperglycemia following a given dose of glucose or the amount of carbohydrates which can be administered without causing glycosuria. It denominates in general the ability of an individual to handle carbohydrates. A high tolerance is the condition where the hyperglycemia after glucose administration is small or transitory, a low tolerance where the hyperglycemia is high or prolonged.

The glucose tolerance tests were developed through the early investigations of Mac Gregor as well as Rollo and Ambrosini (cit. Bailey 1919, Bang 1913, Jacobsen 1913, Allen 1913, Hopkins 1915). They differ mainly in the method of administration of the dextrose: orally or intravenously, one dose or several doses.

A. *One-Dose Oral Dextrose Tolerance Tests.* The dextrose is given as a standard amount: 50 g (Himsworth 1935, Langner and Dewees 1942), or 100 g (Janney and Isacson 1918). The dextrose can be given in an amount proportional to the body weight (Freeman et al. 1942): 1.75 g/kg body weight (Hamman and Hirschmann 1917), and 1 g per kg (Hagedorn 1921, Holst 1922—23, Enochsson 1932). Concentrations higher than 25 % often cause nausea (Ross and Tonk 1938).

Table 1.

*Criteria of the Normal, 2-Dose, 1-Hour Dextrose Tolerance Test,
Given by Previous Authors.*

Author	Blood Sample	Highest Blood Sugar Value in mg %		
		Fasting (F. V.)	At 30 Min. (30 M. V.)	At 60 Min. (60 M. V.)
Exton and Rose (1931)	Venous	—	75 above F. V.	5 above 30 M. V.
Gould et al. (1937)	Venous	120	50 above F. V.	30 above 30 M. V.
Cooperstock and Galloway (1938). In children	Capillary	—	—	80 above F. V.
Matthews et al. (1939)	Venous	120	—	158 (158—179 doubtful, >180 abnormal)
Deweese and Langner (1942)	Capillary	—	—	180
Schmidt and Christensen (1946)	Venous = Matthews et al. Capillary	—	—	170 (170—195 doubtful)

Larger doses of dextrose (above 50 g) increase the duration but not the height of the hyperglycemic reaction; the hyperglycemic effect of dextrose is not proportional to the amount of dextrose given (Hansen 1923, Maclean and De Wesselow 1921, Tisdall et al. 1925).

Without going into details one can briefly say that the following standards of the tolerance curve have been widely accepted: a fasting value less than 120 mg%, a peak below 170 mg% (venous blood) or 200 mg% (capillary blood), a return to the fasting value within $1\frac{1}{2}$ — $2\frac{1}{2}$ hours (cf. Hagedorn 1921, Enocksson 1932, Myers and McKean 1935, Ross and Tonk 1938, Langner and Dewees 1942). Increasing the dose of dextrose from 1.0 to 1.5 per kg may delay the return for as much as an hour (Peters and van Slyke 1946). The curve then often shows a short hypoglycemic phase. Glycosuria does not occur in healthy subjects.

Table 2.
Intravenous Glucose Tolerance Tests.

Author	Cases	Amount of Dextrose in g	Conc. %	Volume in ml	Duration of Injection
Jørgensen and Plum (1923—26)	92	20	20	50	2—4 min.
Lennox and Bel-linger (1927)	100	0.33/kg	20		5 min.
McKean et al. (1935)		0.2/kg	50		1.5 min.
Ross and Tonk (1938)	children	5—20	20		2—4 min.
Crawford (1938)	»	0.5/kg	20		20 ml/45 sek.
Tunbridge and Allibone (1940)	12	27.6	30	92	3 min.
Lozner et al. (1941)	60	25	50	50	2 min.
Greville (1943)	35	0.5/kg 15/m ² body surface	33.3		2 min.

B. The Two-Dose, One-Hour Dextrose Tolerance Test (the Exton-Rose Procedure).

This method takes advantage of the fact known as Hamman-Hirschmann's or Staub-Traugott's phenomenon: after successive doses of dextrose given by mouth each successive rise in the blood sugar curve is less than the preceeding one (Maclean and De Weselow 1921, Hansen 1923, Lennox 1927, Hale-White and Payne 1926, Himsworth 1935, Soskin et al. 1934, 1944, 1946.)

Table 1 gives the criteria of the 2-dose, 1-hour dextrose tolerance test in healthy subjects.

C. Intravenous Dextrose Tolerance Tests. The numerous methods vary above all as regards concentration and amount of solution used and duration of the injection (table 2).

The fasting value is usually reached after 40—60 minutes. Lozner et al. (1941) consider the 2-hour value especially important: it should in healthy subjects not exceed 100 mg% and should in diabetics exceed 120 mg%.

General Considerations of Dextrose Tolerance Tests. The preceding diet has a marked influence on the shape of the dextrose tolerance curve. A diet rich in carbohydrates increases the dextrose tolerance, while a diet poor in carbohydrates decreases it (Kageura 1922, Adlersberg and Porges 1926, Sweeney 1927, McClellan and Wardlaw 1932, Himsworth 1932—36, McCullagh and Johnston 1938).

This applies to all dextrose tolerance tests (Kageura 1922, McClellan and Wardlaw 1932, Sweeney et al. 1937, Tunbridge and Allibone 1940, Wayburn and Gray 1942) though the data on the condition in the 2-dose test still are very incomplete.

Studies on the influence of gastro-intestinal factors on the dextrose tolerance curve have shown that the absorption of dextrose by the stomach seems to be negligible (see Magers 1934), that the emptying rate of the stomach has little or no effect on the oral dextrose test (Cori 1925, Hale-White and Payne 1926, Magers 1934), and that the intestinal absorption mainly determines the shape of the tolerance curve (Cori 1925). A hastened intestinal passage changes the course of the curve (Thaysen 1929, Ross and Tonk 1938).

The fasting blood sugar value is on the whole claimed to be the same in capillary and venous blood. After oral administration of dextrose the sugar content of capillary blood rises faster than that of venous blood, and the difference reaches 20—50 mg%; the two curves then approach till they meet at a normal or hypoglycemic level (cf. Hagedorn 1921, Foster 1923, Gilbert et al. 1926, Friedenson et al. 1928, Cavett and Seljeskog 1934, Marble et al. 1939). Schmidt and Christensen (1946) have found the capillary blood sugar values to be 10 % higher on an average and therefore correct venous blood sugar values by 10 %.

Several authors have made a comparison of different dextrose tolerance tests. Lennox and Bellinger (1927) found a good agreement between the intravenous and the one-dose test but the intravenous test showed less variability. Kelly et al. (1935) as well as Brown (1939) found the 2-dose and 1-dose oral tests to be equally accurate. Young (1937) and Langner and Dewees (1942) consider the one-dose test more accurate. The latter state the 2-dose test to be too sensitive. Schmidt and Christensen (1946) who performed the one-dose as well as two-dose oral tests in the same healthy subjects and in patients, stress the value of the two-dose test for clinical application.

Information is gathering about the separate tolerance tests mentioned, but a comparative investigation of all three tests in the same individual is lacking. The aim of this work is to perform the one-dose and the two-dose oral and the intravenous tolerance tests in one and the same individual and to submit the results to statistical analysis and to evaluate:

- 1) the variability of the single tests;
- 2) the effect of excessive carbohydrate diet;
- 3) a more detailed analysis of the two-dose test as regards the tolerance in a) elderly people, b) subjects confined to bed for a long time and c) patients with chronic infections.

Own Investigations.

Methods.

The tolerance tests were always started at 8 a.m. The subjects fasted from the previous night and were kept resting during the test.

Method A. One-Dose Oral Test. One g dextrose per kg body weight in a 20 % solution. Capillary blood samples 0, 30, 45, 60, 120, 150 and 180 minutes after ingestion of the dextrose solution. Urine analyses at 0, 60, 120 and 180 minutes.

Method B. Two-Dose, One-Hour Oral Test. Fifty g of dextrose in 300 ml water at fasting and after 30 minutes. Capillary and venous blood samples at 0, 30 and 60 minutes. Urine analysis at 60 minutes.

Method C. Intravenous Test. 100 ml of a 25 % solution of dextrose in water intravenously. Time of injection 1 minutes. Blood samples (capillary) at 0, 10, 15, 25, 35, 45, 60 and 90 minutes after end of the injection. In a second series blood-samples also at 120, 150 and 180 minutes. Urine sample after end of the test.

Blood sugar was determined according to Hagedorn-Jensen, the urine analyses according to Benedict.

All three tests were performed on one and the same individual, 32 healthy subjects in all, 20—33 years of age, 22 men and 10 women. None of these had shown any signs of infections or gastrointestinal disorders the month before the test. Only such persons were tested who for a month previous to the test had lived on a diet which contained an approximately normal amount of carbohydrates (200—300 g) daily.

Limits of Variation.

A range of variation of $\pm 2\sigma$ comprises 95.5 % of a normally distributed material. This means that only one case out of 45 will be found above this border. This range can be denoted »the normal

range of variation». In the present investigation σ denotes the variation between individuals and includes the error of the method which is negligible in relation to the individual variation.

We suggest the following procedure for evaluating a tolerance test:

1. A value falling between the borders $M \pm 2\sigma$ is considered as normal.

2. A value found *around* the $\pm 2\sigma$ border may mean one of two things:

a) the value is normal and has by chance fallen at one end of the normal range of variation; in this case a repeated test will most probably give a value which falls closer to the average;

b) the value is abnormal; in that case a repeated test will give a value of the same magnitude or definitely falling outside the border.

The conclusion will be that a value falling on the 2σ borders indicates that the test should be repeated. If the second test now gives a normal value, the test will be considered as normal and the value found in the first test due to chance (a). If the second test, however, gives a value falling around or outside the 2σ -border, the test is probably abnormal (b).

3. The further a value differs from the 2σ -border the more likely that the value is abnormal. Values around the 2.5σ -borders which comprise 98.8 % of the material, are very probably abnormal. This means that only one normal value out of 167 falls *above* this border.

4. Values falling around the 3.3σ -borders which comprise 99.9 % of the material are definitely abnormal. This means that only one case out of 2,000 falls *above* this border.

The curves are evaluated according to these principles, and in order to show the agreement between the calculated normal range of variation ($M \pm 2\sigma$) and the range found, both are tabulated. The close agreement justifies the principles stressed above and is proved by the following. The range of variation of a material is connected with the number of cases (Tippett 1926): a material of 32 individuals has a theoretical range of $\pm 2.07\sigma$, thus an agreement within 3—4 % with the range found.

The upper limits of variation for all tests are condensed in table 11 (page 219).

A way of comparison by using σ -units is shown in a second paper (Goldberg and Luft 1949 [in press]).

Results.

Fasting Blood Sugar Values. As all three tests were performed on one and the same individual on different days, the variability of the fasting value of an individual from day to day can be calculated. The 96 fasting blood sugar values in 32 individuals varied between 64 and 120 mg%, 96.0 mg% on an average. The mean difference from day to day was $-1.4 \text{ mg}\% \pm 3.0$, thus not significant.

By the method of double determinations the variation of the difference from day to day (σ_d) was determined and found to be 17.2 mg% ($n = 32$), corresponding to 17.8 % of the mean. It can thus be calculated that the variability of a single value in one individual from day to day (σ_s) is

$$\sigma_s = \frac{1}{\sqrt{2}} \cdot \sigma_d = 12.1 \text{ mg}\%,$$

corresponding to 12.5 % of the mean.

The variability of the fasting blood sugar value of a single individual is thus of the same magnitude as the variation of the fasting blood sugar value between different individuals: 11.4, 11.4 and 12.8 mg% (cf. the variability of the fasting values in tables 3, 4 and 9).

A. One-Dose Oral Tolerance Test.

The blood sugar curves after ingestion of dextrose agreed completely in men and women. An average curve for the whole material and the normal range of variation ($\pm 2\sigma$) are given in fig. 1.

The values, their variations and the range are given in table 3.

It is seen from curve 1 and table 3 that the largest absolute blood sugar variations are found around the maximum value. The maximum value, 153 mg% on an average, varies from 116—210 mg%. The variations of the blood sugar are less in the two-hour and later values.

The same tendency is seen when the variations are expressed in per cent of the mean: the highest variation around the maximum and the lowest at the end of the curve.

The average of the maximal increase of the blood sugar above the fasting value for each single individual was 56.9 mg% and varied within a wide range, from 17 to 94 mg%.

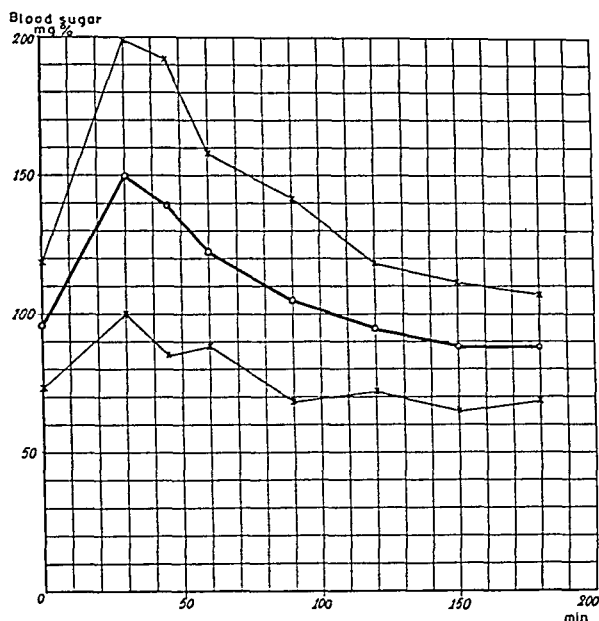


Fig. 1. Oral One-Dose Dextrose Tolerance Test.

○—○ average curve for 32 healthy subjects,
 ×—× normal range of variation comprising 19 cases out of 20 ($\pm 2\sigma$).

It can be discussed if the maximal increase of a single curve above the fasting value is related to the height of the fasting value. Variyar and Mayar (1946) have claimed that the blood glucose values noted after oral administration of dextrose vary directly with the fasting blood sugar values and found a correlation coefficient of 0.51.

We have correlated the maximal *increase* above the fasting value to the fasting value and found a correlation coefficient of 0.10 ± 0.17 , thus no correlation at all. The increase is not correlated to the fasting value, at least not in healthy subjects. Variyar's and Mayar's findings are due to an erroneous way of correlating their data.

The curve returns to the fasting value within 58—180 minutes, 109 minutes on an average. This variation is very considerable and larger than that earlier accepted as standard (cf. page 202). In a number of cases the blood sugar curve fell rather rapidly to a value slightly higher than the fasting value, then run horizontally for a certain period of time, whereafter came the decline to a hypoglycemic level. We have for practical reasons determined the time of return to the fasting value and the return to a value

Table 3.

Oral One-Dose Dextrose Tolerance Test in 32 Healthy Subjects.

Time in minutes	Mean $\pm \epsilon_M$ mg %	Standard Deviation (σ)		Range	
		mg %	Per Cent of Mean	Found	Calculated ($M \pm 2 \sigma$)
0.....	95.9 \pm 2.0	11.4	11.9	78—120	73—119
30.....	149.9 \pm 4.4	24.6	16.4	108—210	101—199
45.....	139.2 \pm 4.9	26.9	19.3	96—180	85—193
60.....	123.3 \pm 3.1	17.5	14.2	78—206	88—158
90.....	105.3 \pm 3.3	18.5	17.6	73—167	68—142
120.....	95.0 \pm 2.0	11.5	12.1	78—121	72—118
150.....	87.8 \pm 2.0	11.4	13.0	70—120	65—111
180.....	87.7 \pm 1.7	9.5	10.9	66—106	69—107
Maximal value mg %	153.4 \pm 5.5	30.9	20.1	116—210	92—215
Increase above fasting value mg %	56.9 \pm 3.7	21.0	37.0	17— 94	15— 99
Time to fasting value	109.2 \pm 5.9	31.7	29.0	58—180	46—173
Time to fasting value (+ 10 mg %)	85.2 \pm 5.0	28.1	33.0	45—145	29—141

within 10 mg% above the fasting value. The curve returns to the fasting value (+ 10 mg%) within 45—145 minutes, 85 minutes on an average.

We suggest the following as standards for the tolerance curve in healthy subjects:

a maximal value, not exceeding 215 mg%; values around 215 should indicate repetition of the test, while values between 215—255 are very likely to be abnormal;

or an increase above the fasting value not exceeding 99 mg%; values between 100 and 126 are very likely to be abnormal;

a return to the fasting value (+ 10 mg%) within 141 minutes; values between 141—176 minutes are very likely to be abnormal.

Glycosuria did not occur in any of the oral one-dose tolerance tests.

B. *The Two-Dose, One-Hour Tolerance Test.*

The blood sugar curves for men and women during the two-dose test agreed completely, for venous as well as for capillary

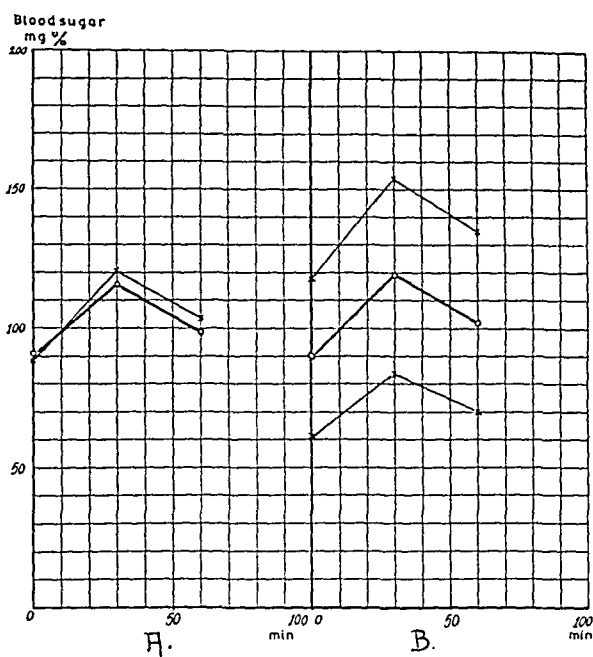


Fig. 2. Oral Two-Dose, One-Hour Dextrose Tolerance Test (*Venous Blood*).

- A. \times — \times average curve of 22 healthy men.
 \circ — \circ , , , 10 , women.
 B. \circ — \circ average curve for 32 healthy subjects.
 \times — \times normal range of variation comprising 19 cases out of 20 ($\pm 2\sigma$).

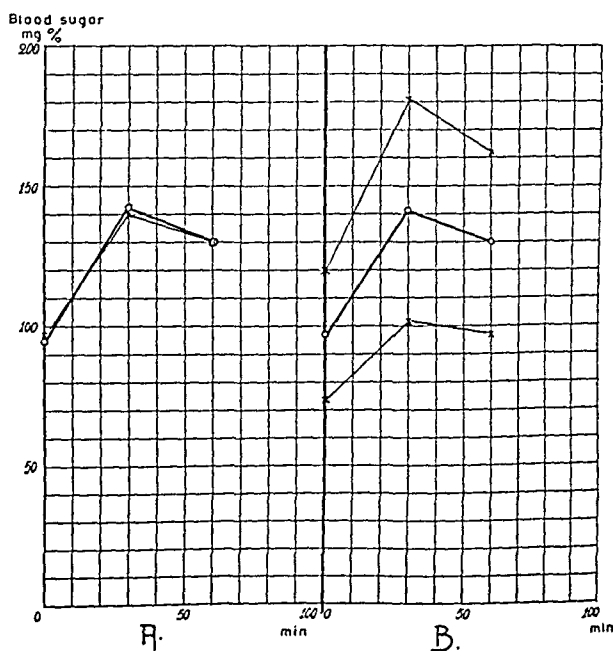


Fig. 3. Oral Two-Dose, One-Hour Dextrose Tolerance Test (*Capillary Blood*).

- A. \times — \times average curve of 22 healthy men.
 \circ — \circ , , , 10 , women.
 B. \circ — \circ average curve for 32 healthy subjects.
 \times — \times normal range of variation comprising 19 cases out of 20 ($\pm 2\sigma$).

Table 4.

Oral Two-Dose, One-Hour Dextrose Tolerance Test in 32 Healthy Subjects.

Time in minutes	Mean $\pm \epsilon_M$ mg %	Standard Deviation (σ)		Range	
		mg %	Per cent of Mean	Found mg %	Calculated ($M \pm 2 \sigma$)
<i>A. Venous Blood.</i>					
0.....	89.7 \pm 2.4	13.8	15.1	56—115	62—117
30.....	119.5 \pm 3.1	17.8	14.9	84—147	84—155
60.....	102.7 \pm 2.9	16.3	15.9	73—140	70—135
30'—0'.....	29.9 \pm 2.3	13.1		+ 6—+57	+ 4—+56
60'—0'.....	12.4 \pm 3.1	17.6		—33—+42	—23—+48
60'—30'.....	—16.8 \pm 1.9	10.8		—49—+ 0	—38—+ 5
<i>B. Capillary Blood.</i>					
0.....	96.8 \pm 2.0	11.4	11.8	64—120	74—120
30.....	141.8 \pm 3.5	19.6	13.8	105—184	103—181
60.....	129.7 \pm 3.0	16.9	13.0	100—170	96—164
30'—0'.....	44.9 \pm 2.8	16.0		+15—+88	13— 87
60'—0'.....	32.9 \pm 2.9	16.4		+ 4—+57	0— 66
60'—30'.....	—12.0 \pm 2.3	13.2		—65—+ 5	—38—+14

blood (figs. 2 A and 3 A). In figs. 2 B and 3 B the average curves of the whole material and the normal range of variation are given. The average values and the variability are shown in table 4.

Venous Blood.

The average of 0, 30 and 60 minutes values as well as the differences between single blood sugar values are given in table 4.

A statistical evaluation of our data gives the following criteria of the normal two-dose test (venous blood):

a 30 minute value not above 155 mg% or an increase from the fasting value less than 56 mg%;

a 60 minute value not above 135 mg%, an increase above the fasting value not exceeding 48 mg% or an increase above the 30-minute value not exceeding 5 mg%.

In comparison with the one-dose test this procedure shows a less variability whether one discusses the single values or the differences (table 4).

Glycosuria occurred in only two cases out of 32. These two cases,

Table 5.

Two-Dose, One-Hour Dextrose Tolerance Test. Difference between Capillary and Venous Blood Sugar Values in 32 Healthy Subjects.

Time in minutes	Difference $\pm \epsilon_D$ mg %	Difference in Per Cent of Venous Blood Sugar %	Standard Deviation (σ) mg %	Range Found mg %
0.....	+ 7.2 \pm 1.8	8.0	10.0	- 7 + 34
30.....	+ 22.2 \pm 2.1	18.6	12.1	\pm 0 + 42
60.....	+ 27.0 \pm 2.2	26.3	12.6	+ 5 + 53

however, showed a very slight glycosuria, amounting to 0.3 and 0.2 g respectively.

Capillary Blood.

The averages of the 0, 30 and 60-minutes values and differences between single blood sugar values are given in table 4. The statistical evaluation of our data gives the following criteria of the normal two-dose test (capillary blood):

a 30-minute value not exceeding 181 mg% or an increase from the fasting value less than 77 mg%;

a 60-minute value not above 164 mg%, an increase above the fasting value not exceeding 66 mg% or an increase above the 30-minute value not exceeding 9 mg%.

A few previous authors consider the capillary tests less reliable. (Deweese, Langner 1942 a. o.). A comparison between the capillary and venous blood sugar values in our material shows that the variability is the same or even slighter in capillary blood samples. Our investigations in healthy subjects thus show a definite reliability of the capillary test.

The Capillary-Venous Blood Sugar Difference. The capillary venous difference (table 5) was found to be 7.2 mg% \pm 1.8 for fasting values, thus a slight but significant difference. The difference increased during the test and was at 30 minutes 22.2 mg% \pm 2.1 on an average and at 60 minutes 27.0 mg% \pm 2.2. The differences varied between - 7 and + 53 mg%.

The capillary-venous blood sugar difference thus increases from the fasting value, not only absolutely from 7 to 27 mg% on an average, but more strikingly if related to the prevailing venous blood sugar concentration, the relative difference increasing from

Table 6.

*Oral Two-Dose, One-Hour Dextrose Tolerance Test in Healthy Subjects
41—80 Years of Age.*

Time in minutes	Mean $\pm \epsilon_M$ mg %	Standard Deviation (σ)		Range	
		mg %	Per Cent of Mean	Found mg %	Calculated ($M \pm 2 \sigma$)
<i>A. Venous Blood.</i>					
0.....	86.1 \pm 2.2	19.1	10.5	76—104	68—105
30.....	124.5 \pm 5.8	24.0	19.3	85—168	77—173
60.....	106.5 \pm 5.9	24.2	22.7	66—140	58—155
30'—0'.....	38.1 \pm 5.2	21.2		0—+53	—4—+80
60'—0'.....	20.1 \pm 5.6	22.9		—13—+72	—26—+66
<i>B. Capillary Blood.</i>					
0.....	91.7 \pm 1.9	7.9	8.9	77—107	76—108
30.....	144.8 \pm 1.9	14.3	9.9	115—170	116—173
60.....	138.9 \pm 4.3	17.8	12.8	113—167	103—175
30'—0'.....	53.1 \pm 3.5	14.6		+24—+72	+24—+82
60'—0'.....	47.2 \pm 4.6	19.0		+8—+77	+9—+85

8 % in the fasting values to 18.6 % at 30 minutes and 26.3 % at 60 minutes.

In spite of the decrease of the blood sugar values from 30 to 60 minutes there was thus a statistically significant increase of the capillary-venous difference.

Two-Dose Test in High Age. In order to establish the variations of the oral two-dose test in higher age this test was performed in 17 healthy subjects, 41—80 years of age. The results are given in table 6.

The average values both for venous and capillary blood are slightly higher than the normal values (table 4), the differences, however, in general not being significant. The only probable difference from normal was the 60—0 minute values for the capillary tests: 14.3 ± 5.5 ($t = 2.6$; $df = 16$; $P = 0.02$) (t-analysis according to Fisher 1936). This difference suggests a slightly lower dextrose tolerance in higher age. In these subjects having a lower fasting value the difference 60'—0' was the most reliable test.

This result is in accordance with earlier investigations with
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Table 7.

Oral Two-Dose, One-Hour Dextrose Tolerance Test in 6 Patients without Infections Confined to Bed for More Than 3 Months.

Time in minutes	Mean $\pm \epsilon_M$ mg %	Standard Deviation (σ)		Range	
		mg %	Per Cent of Mean	Found	Calculated ($M \pm 2 \sigma$)
<i>A. Venous Blood.</i>					
0.....	82.3 \pm 4.1	10.0	12.1	70—100	62—102
30.....	121.8 \pm 4.1	10.1	8.3	104—134	102—142
60.....	120.8 \pm 4.9	12.1	10.0	100—134	97—145
30—0.....	39.5 \pm 3.7	9.1		28— 55	21— 57
60—0.....	38.5 \pm 8.2	20.2		0— 53	0— 78
<i>B. Capillary Blood.</i>					
0.....	85.0 \pm 3.5	8.7	10.2	77—101	68—102
30.....	134.8 \pm 3.8	9.3	6.9	117—143	116—153
60.....	146.0 \pm 8.7	21.4	14.7	110—170	103—189
30—0.....	48.2 \pm 4.8	11.7		30— 63	25— 71
60—0.....	61.0 \pm 11.4	28.0		9— 85	5—116

the two-dose test (Matthews et al. 1939) and the one-dose oral method, which show that the height and duration of alimentary hyperglycemia are increased in old age (Punschel, 1923, Hale-White and Payne 1926, Aaltonen 1939).

Two-Dose Tests in Patients confined to Bed. Without Infections. The tests were performed on six patients confined to bed for more than three months because of skeletal fractures uncomplicated by infections. The results are shown in table 7.

For both the venous and capillary test the 30-minutes values fell within the normal range of variation, while the 60-minute values very probably were higher than normal (cp. table 4); the 30'—0' difference was probably higher than normal, while the 60'—0' was significantly higher.

With Chronic Infections. The tests were performed in six patients with tuberculous spondylitis that was considered almost cured. The patients were confined to bed for more than three months. They had no fever and no-increased sedimentation rate, no fistulas or signs of active processes on X-ray. The results of the tolerance tests are shown in table 8 (only capillary tests):

Table 8.

*Oral. Two-Dose, One-Hour Dextrose Tolerance Test (Capillary Blood)
in 6 Patients with Chronic Infection, Confined to Bed for
More Than 3 Months.*

Time in minutes	Mean $\pm \epsilon_M$ mg %	Standard Deviation (σ)		Range	
		mg %	Per Cent of Mean	Found	Calculated ($M \pm 2 \sigma$)
0.....	94.7 \pm 3.3	9.3	9.9	78—106	76—113
30.....	159.2 \pm 5.7	13.9	8.7	147—178	131—187
60.....	181.8 \pm 7.5	18.4	10.1	159—213	145—219
30—0.....	64.5 \pm 8.0	19.5		47—100	25—103
60—0.....	87.2 \pm 10.0	24.4		65—135	38—136

Both the 30- and 60-minute values were significantly higher than the normal average, likewise the differences 30'—0' and 60'—0' (cp. table 4).

C. The Intravenous Dextrose Tolerance Test.

The blood sugar curves agreed for men and women during the intravenous test. In fig. 4 the average curve of the whole material is given and the variability is shown in table 9.

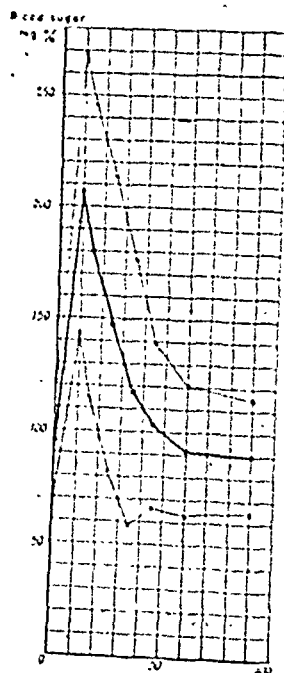


Fig. 4. Intravenous Dextrose Tolerance Test (90 min.).
○—○ average curve for 32 healthy subjects.
x—x normal range of variation comprising
19 cases out of 20 ($\pm 2 \sigma$).

Table 9.

Intravenous Dextrose Tolerance Test (90 Min.) in 32 Healthy Subjects.

Time in minutes	Mean $\pm \epsilon_M$ mg %	Standard Deviation (σ)		Range	
		mg %	Per Cent of Mean	Found mg %	Calculated ($M \pm 2 \sigma$)
0.....	96.1 \pm 2.3	12.8	13.5	70—120	71—122
10.....	206.8 \pm 5.5	31.3	15.2	144—289	144—269
15.....	180.3 \pm 5.7	32.5	18.0	108—256	115—245
25.....	147.4 \pm 5.8	32.8	22.3	86—200	82—213
35.....	117.1 \pm 5.2	29.3	25.0	50—174	59—176
45.....	102.7 \pm 3.2	17.6	18.1	70—130	67—138
60.....	92.5 \pm 2.5	14.1	19.3	63—122	64—121
90.....	90.2 \pm 2.2	12.6	14.0	62—114	65—115
Maximal value mg %	207.8 \pm 5.7	32.4	15.6	144—298	143—273
Time to fasting value	55'.6 \pm 3.4	19'.4	34.9	23'— 90'	17'— 94'

In 15 healthy subjects an intravenous tolerance test was made and blood sugar values were determined up to 180 minutes after the injection. The average curve during the first 90 minutes coincided completely with that already given in fig. 4. The variability is given in table 10.

As in the one-dose oral test the variability of the blood sugar value is largest around the peak of the blood sugar curve. Contrary to the oral test the largest variation is not found at the maximum point (at 10 minutes) but 15—45 minutes after the injection (table 9).

The maximum blood sugar value varies not only with the individual tolerance but also with the rate of injection and the total amount of sugar administered per kg body weight.

The time for return of blood sugar to the fasting value varied considerably, between 23 and 90 minutes.

The height of the blood sugar level at 45 and 90 minutes respectively varied between 70—130 mg% and 62—114 mg%.

So far it seems likely that a return to the fasting blood sugar value within 94 minutes, a 45-minute value not exceeding 138 mg% and a 90-minute-value not exceeding 115 mg% might be used as criteria for the upper limits of the normal curve.

Table 10.

Intravenous Dextrose Tolerance Test (180 Min.) in 15 Healthy Subjects.

Time in minutes	Mean $\pm \epsilon_M$ mg %	Standard Deviation (σ)		Range	
		mg %	Per Cent of Mean	Found mg %	Calculated ($M \pm 2 \sigma$)
0.....	93.1 \pm 1.6	6.1	6.5	85—103	81—105
10.....	205.0 \pm 5.9	22.8	11.1	174—256	159—251
15.....	180.7 \pm 6.9	26.5	14.7	119—228	128—234
25.....	145.3 \pm 6.6	25.7	17.7	94—200	94—197
35.....	114.1 \pm 5.9	22.8	20.0	89—172	69—160
45.....	103.8 \pm 5.6	21.6	20.8	77—158	61—147
60.....	94.3 \pm 3.9	15.2	16.1	78—136	64—125
90.....	90.5 \pm 2.4	9.2	10.2	74—107	72—109
120.....	88.9 \pm 1.7	6.6	7.4	81—105	76—102
150.....	89.6 \pm 2.0	7.7	8.6	75—104	74—105
180.....	86.9 \pm 1.9	7.2	8.3	75—101	73—101

Glycosuria occurred during the test in 25 out of 32 cases. The absolute amount of glucose excreted was 0.1—1.0 g or 0.36 g \pm 0.04 ($\sigma = 0.24$, $n = 25$).

D. *The Effect of Excessive Carbohydrate Diet on the Dextrose Tolerance Curve.*

In seven healthy subjects the three different dextrose tolerance tests were performed twice: the first time after a period on a normal diet containing a normal amount of carbohydrates (200—300 g daily), the second after seven days on a diet, where the amount of carbohydrates was increased by substituting parts of the former diet with increased amounts of bread and potatoes and adding to it 100 g of sugar daily. This would correspond to a total amount of carbohydrates of 350—500 g daily. The results are given in fig. 5.

No significant difference was seen for any of the tests.

It is well known that a diet insufficient in carbohydrates will diminish the dextrose tolerance (page 204). On the other hand it is obvious from our experiments that all three dextrose tolerance curves had the same course whether the diet was sufficient or excessively rich in carbohydrates.

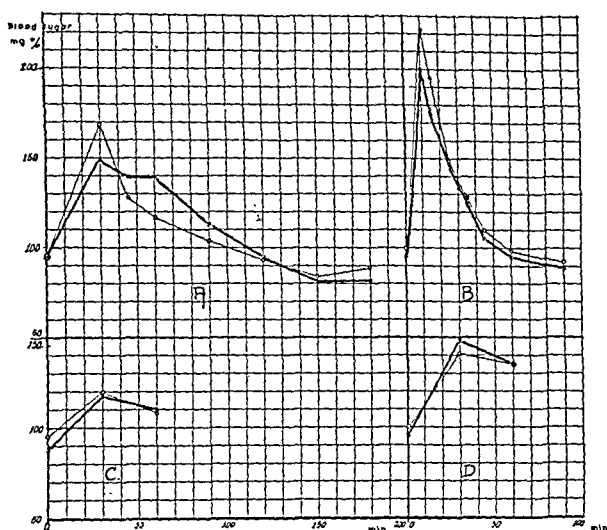


Fig. 5. Effect of Excessive Carbohydrate Diet.

- A. Oral One-Dose Test.
 B. Intravenous Test.
 C. Oral Two-Dose Test (Venous Blood).
 D. Oral Two-Dose Test (Capillary Blood).
 × — × on normal diet.
 ○ — ○ on excessive carbohydrate diet.

E. Implications.

For practical reasons the upper limits of variation have been condensed in the following table (no. 11): the normal ($M + 2\sigma$), the probably abnormal ($M + 2.5\sigma$) and the abnormal ($M + 3.3\sigma$) limit.

The practical applications of the different limits tabulated have been discussed on page 205.

A comparison of the variability from individual to individual of the two-dose, one-hour test with the variability of the oral one-dose as well as the intravenous test shows the two-dose test consistently to have a less variability than the other two methods. This implies a greater reliability of the two-dose test and also makes it probable that this test will be more sensitive in distinguishing cases with pathological dextrose tolerance.

The advantages of the two-dose oral test of essential importance to clinical application are:

- a) a short duration of the test (one hour);
- b) simplicity in performance: only three blood samples and capillary blood being as reliable as venous blood;

Table 11.
Criteria of Dextrose Tolerance Tests.
(32 cases.)

Dextrose Tolerance Tests. (32 cases.)							
	Mean $\pm \epsilon_M$ mg %	Standard Deviation (σ)		Upper Limits of Variation			
		mg %	% of Mean	>Normal, M + 2 σ	>Probably Abnormal, M + 2.5 σ	>Ab- normal, M + 3.3 σ	
I. Oral One-Dose Test.							
Maximum:....	153.4 \pm 5.5	30.9	20.1	215	231	255	
Increase over fasting:	56.9 \pm 3.7	21.0	37.0	99	109	126	
Time to fast- ing:.....	109'.2 \pm 5.9	31'.7	29.0	173'	189'	214'	
Time to fasting (+ 10 mg %):	85'.2 \pm 5.0	28'.1	33.0	141'	156'	178'	
Glycosuria....	absent						
II. Oral Two-Dose One Hour-Test.							
A. Venous Blood.							
0'.....	89.7 \pm 2.4	13.8	15.4	117	124	135	
30'.....	119.5 \pm 3.1	17.8	14.9	155	164	178	
60'.....	102.7 \pm 2.9	16.3	15.9	135	144	156	
Diff. 30'— 0'.	29.9 \pm 2.3	13.1		56	63	73	
> 60'— 0'.	12.4 \pm 3.1	17.6		48	56	70	
> 60'—30'.	-16.8 \pm 1.9	10.8		5	10	19	
Cap-ven. diff. 60'.....	27.0 \pm 2.2	12.6		52	58	69	
B. Capillary Blood.							
0'.....	96.8 \pm 2.0	11.4	11.8	120	125	134	
30'.....	141.8 \pm 3.5	19.6	13.8	181	191	206	
60'.....	129.7 \pm 3.0	16.9	13.0	164	172	185	
Diff. 30'— 0'.	44.9 \pm 2.8	16.0		77	85	98	
> 60'— 0'.	32.9 \pm 2.9	16.4		66	74	87	
> 60'—30'.	-10.9 \pm 1.7	9.7		9	13	21	
Glycosuria....							
2 cases out of 32 (0.2 and 0.3 g resp.)							
III. Intravenous Test.							
45'.....	102.7 \pm 3.2	17.6	18.1	138	147	161	
90'.....	90.2 \pm 2.2	12.6	14.0	115	122	132	
Time to fast- ing:.....	55'.6 \pm 3.4	19'.4	34.9	94'	104'	120'	
Glycosuria....	0.36 g (25 cases out of 32, range 0.1—1.0 g)						

c) simplicity in evaluation of the test;

d) less variability, which yields a higher degree of reliability.

Definite conclusions concerning the sensitivity of the different tests in distinguishing disturbances of dextrose tolerance can be drawn only after examining a large number of different pathological cases. Investigations have been completed showing the value of the different tests in endocrine disturbances (Goldberg and Luft 1949).

Summary.

A one-dose and two-dose oral as well as an intravenous dextrose tolerance test was performed in 32 healthy subjects (22 males and 10 females).

The limits of variation for all three tests were established and their implications were discussed.

The two-dose oral test consistently showed a less variability than the other two tests, the capillary blood samples being as reliable as venous blood. The advantages for clinical applications were stressed.

All three dextrose tolerance curves had the same course whether the diet was sufficient or excessively rich in carbohydrates.

The limits of variation of all three tests were further established in 17 elderly subjects, 41—80 years of age.

The two-dose, one-hour test was finally performed in patients confined to bed for more than three months.

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On Serum Bilirubin during the Course of an Icterus.

By

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(Submitted for publication January 14, 1948.)

Little is known about the changes undergone by the serum bilirubin in patients with an icterus during the course of the illness. Hijmans van den Bergh (6) had already found that the diazo reaction is often indirect during the onset of an obstructive jaundice. After ligation of the common duct in rabbits we also saw an icterus with indirect reacting bilirubin. Only after 24—28 hours the diazo reaction became direct (2, 8).

Fiessinger (4) discovered that the diazo reaction often slowed down during the decrease of an icterus. He did not explain how this rate was determined. Hartog (5) was in great doubts about the correctness of this observation as he always found a direct diazo reaction. And that was as far as the knowledge about the rate of the diazo reaction during the course of an icterus went.

A little more was known about the adsorption of bilirubin on the albumin precipitate, which occurs when we withdraw albumin from serum, both in case of an increasing and a decreasing icterus.

Meulengracht (10) noticed that the albumin precipitate is far yellower in a decreasing icterus than in an increasing icterus. Other investigators with more accurate methods discovered that the adsorption of bilirubin on the albumin precipitate does increase in a decreasing icterus.

Wiemer (12) observed that the adsorption decreased again in case of a further decrease of an icterus.

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According to Weltmann and Jost we also find different adsorptions of bilirubin on the albumin precipitate in case of strong fluctuations of the bilirubin percentage in a haemolytic icterus in which the bilirubin percentage of the blood is high.

The instance by Weltmann and Jost was not a very felicitous one. A patient with a sepsis lenta and a heart-failure is not a beautiful example of a patient with a hemolytic icterus; especially if the patient's urine contains bilirubin.

My Own Investigations.

From a number of patients with an icterus the rate of the diazo reaction in the serum was determined during the illness on various days. The method described by Deenstra (3) was used and in this method the rate of the diazo reaction is expressed in the »one minute percentages«.

The adsorption of bilirubin on the albumin precipitate was likewise determined in a number of patients (fig. 3—14).

The bilirubin percentage was determined with the method of Hijmans van den Bergh for indirect bilirubin:

4 ml of absolute alcohol is added to 2 ml of serum. After shaking and centrifuging, 0.5 ml of diazo reagent and 1 ml of alcohol are added to 4 ml of the clear filtrate. Then the bilirubin percentage is determined by measuring the extinction of the azobilirubin solution with the aid of the Stufen photometer (filter S 53).

So the quantity of bilirubin which is absorbed on the albumin precipitate, is determined. After that we calculate how many per cent of the bilirubin which is present in the serum, was not adsorbed on the albumin precipitate. A curve was made of these percentages.

Case Reports.

Fig. 1 shows the one minute percentage in a patient with icterus catarrhalis.

Four days after the patient's skin had become yellow, the first examination of the serum took place. The icterus decreased continually after that. The rate of the diazo reaction first increased but then decreased rapidly. After the 27th day, the rate of the diazo reaction increased, however, though the icterus decreased steadily. Only a considerable time afterwards did the rate of the diazo reaction decrease again. The phenomenon, that the rate of the reaction in-

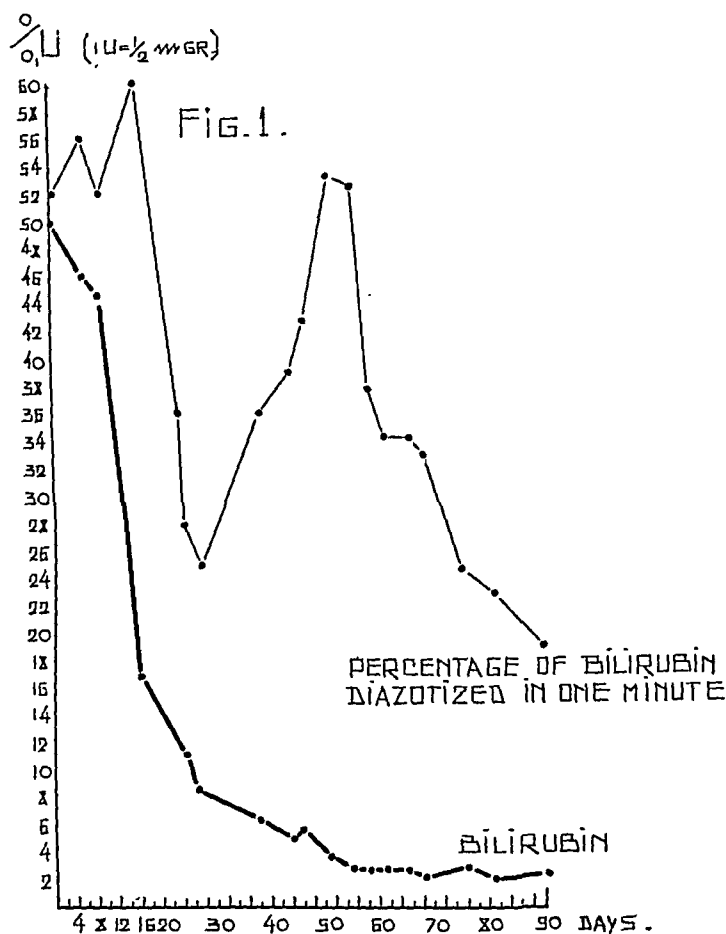


Fig. 1. Bilirubin percentage of the serum and rate of the diazo reaction in a patient with icterus catarrhalis.

creases again after a temporary decrease of this rate, was observed more or less clearly by me in all patients with an icterus catarrhalis.

The following instances are no selected cases.

Fig. 2 shows the examination of a patient with an icterus catarrhalis, whose skin became yellow one week before the first examination of the serum. The icterus had already decreased a little when the serum was examined for the first time. We also see now that the curve, rendering the rate of the diazo reaction, shows two peaks. On the 40th day after the first examination of the serum the icterus increased again. It is very likely that the shape of the second peak is influenced by this recurrence. After this recurrence no more peaks are seen.

Fig. 3. shows the results of the examination of the serum from a nurse with an icterus catarrhalis, whose skin became yellow one day before the first examination of the serum. In this patient the degree of adsorption of bilirubin on the albumin precipitate was also determined every time. We see that during the increase of the icterus, the rate of the diazo reaction increases too. If the icterus decreases,

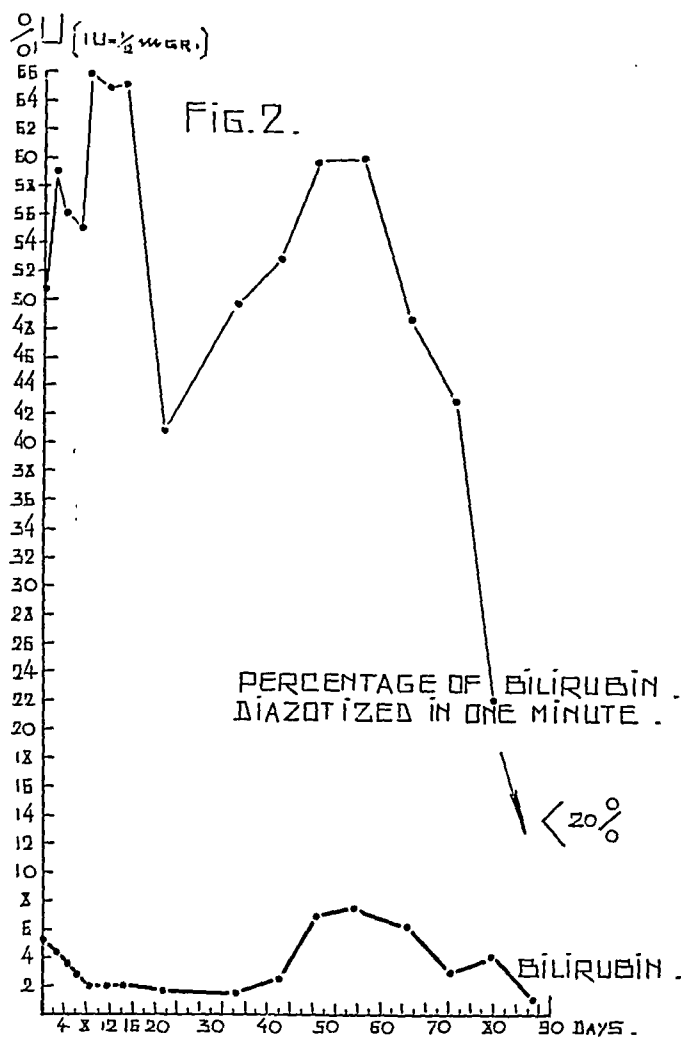


Fig. 2. Bilirubin percentage of the serum and rate of the diazo reaction in a patient with an icterus catarrhalis.

the diazo reaction continues to increase for a little while, but then it slows down. Here too we see a second peak in the curve, rendering the rate of the diazo reaction. This peak, however, is not so pronounced as in the preceding curves.

It strikes us, however, that the bilirubin percentage of the serum rises simultaneously with the increase of the rate of the diazo reaction. The curve rendering the percentage of bilirubin which is not adsorbed on the albumin precipitate proves to be a very remarkable one. If the rate of the diazo reaction decreases, this curve falls, and if the diazo reaction becomes more rapid, this curve rises again. We might get the impression that the rate of the diazo reaction and

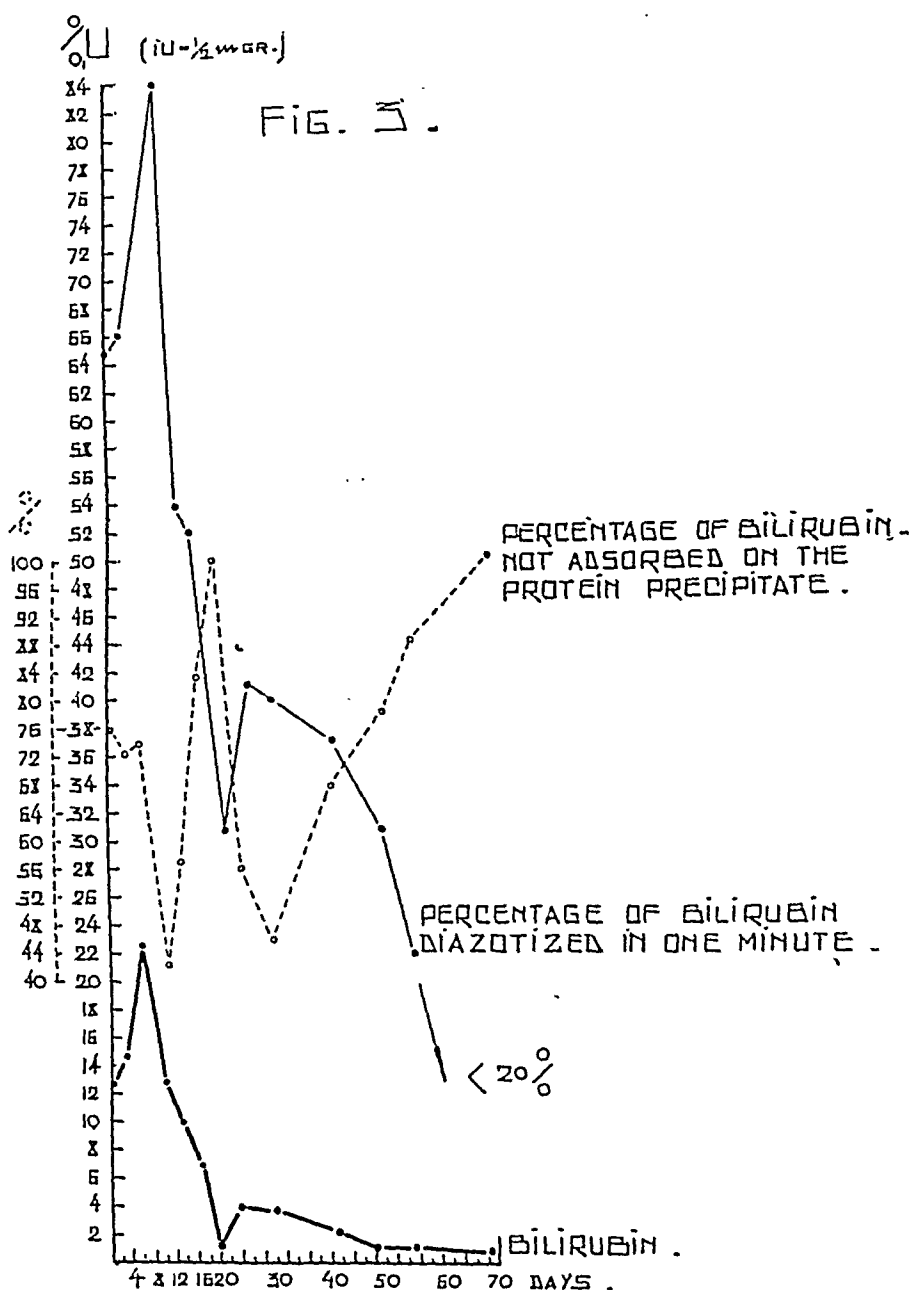


Fig. 3. Bilirubin percentage of the serum, rate of the diazo reaction and percentage of bilirubin which is not adsorbed on the albumin precipitate in a patient with icterus catarrhalis.

the degree of adsorption of bilirubin on the albumin precipitate are directly related to each other. It will be proved later on that the relation is not so pronounced as is suggested by fig. 3.

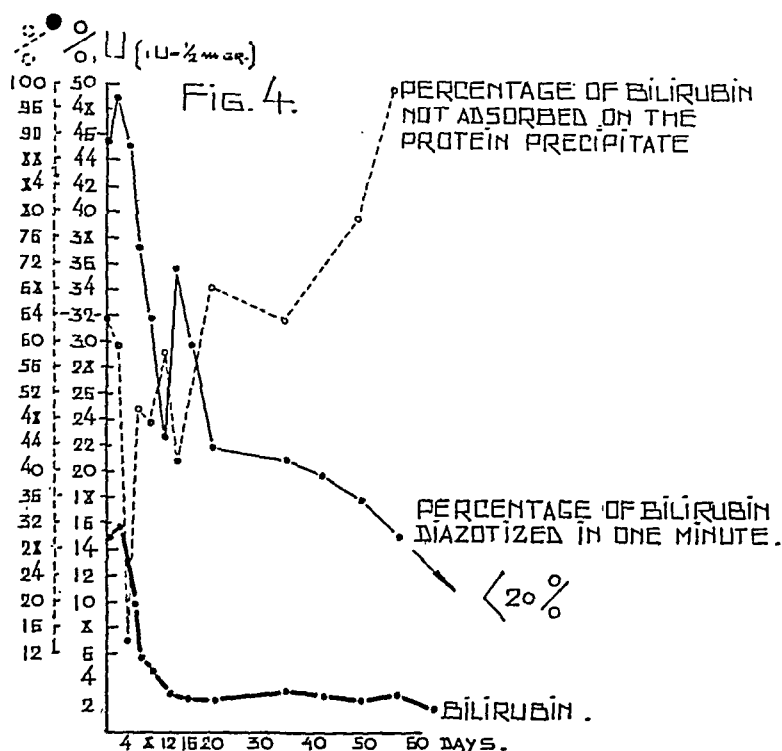


Fig. 4. Bilirubin percentage of the serum, rate of the diazo reaction and percentage of bilirubin which is not adsorbed on the albumin precipitate in a patient with icterus catarrhalis.

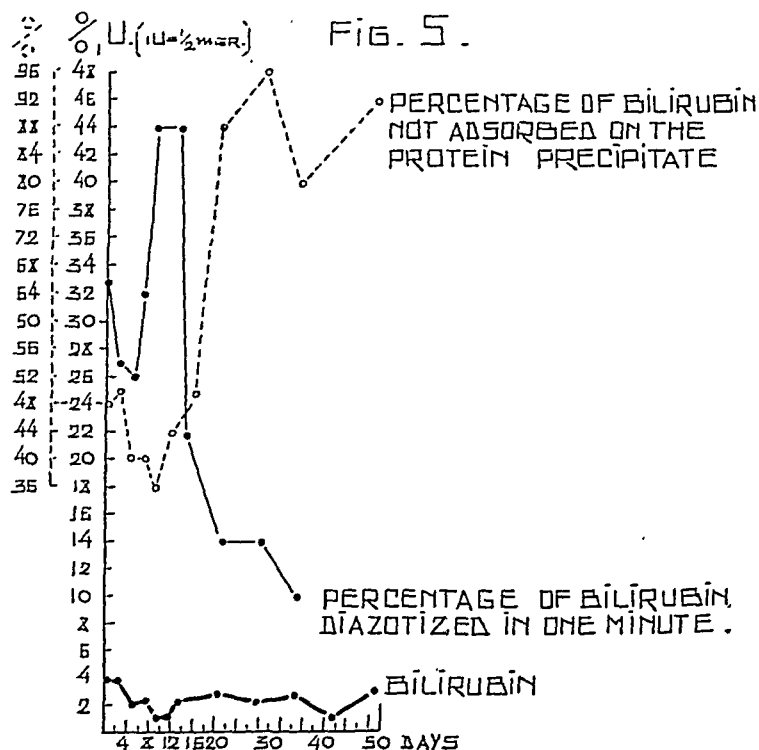


Fig. 5. Bilirubin percentage of the serum, rate of the diazo reaction and percentage of bilirubin which is not adsorbed on the albumin precipitate in a patient with icterus catarrhalis.

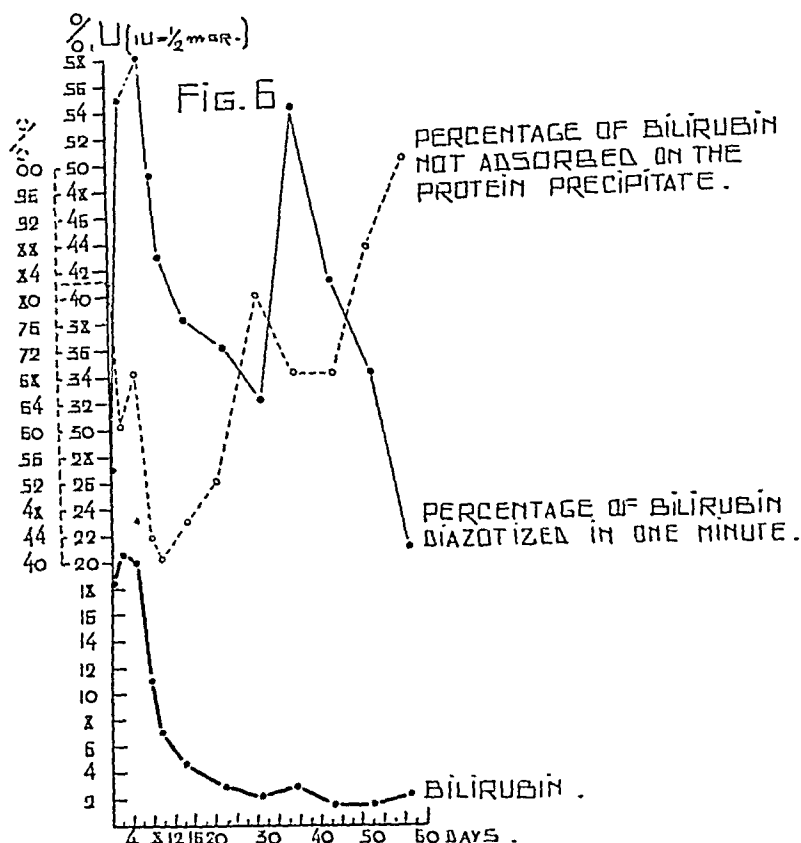


Fig. 6. Bilirubin percentage of the serum, rate of the diazo reaction and percentage of bilirubin which is not adsorbed on the albumin precipitate in a patient with icterus catarrhalis.

Furthermore the observations by Fiessinger, who discovered that the adsorption of bilirubin on the albumin precipitate increases if the icterus decreases, prove to be right. Fiessinger, however, did not have sufficiently accurate methods at his disposal to determine accurately the adsorption on the albumin precipitate and the rate of the diazo reaction. That is the reason why he did not find, after a temporary decrease and increase, that the adsorption of bilirubin on the albumin precipitate decreases, in case of a further decreasing icterus.

(For a clear apprehension of the above argument, I point to the fact that the curve falls in case of an increase of the adsorption of bilirubin on the albumin reagent.)

The curves of the figures 4, 5, 6, 7 and 8 also render the examination of the serum from patients with an icterus catarrhalis. The curves rendering the rate of the diazo reaction and the degree of adsorption on the albumin precipitate are not always each other's image. The point of time on which the adsorption of the bilirubin is maximal often falls somewhat later than the point of time on which

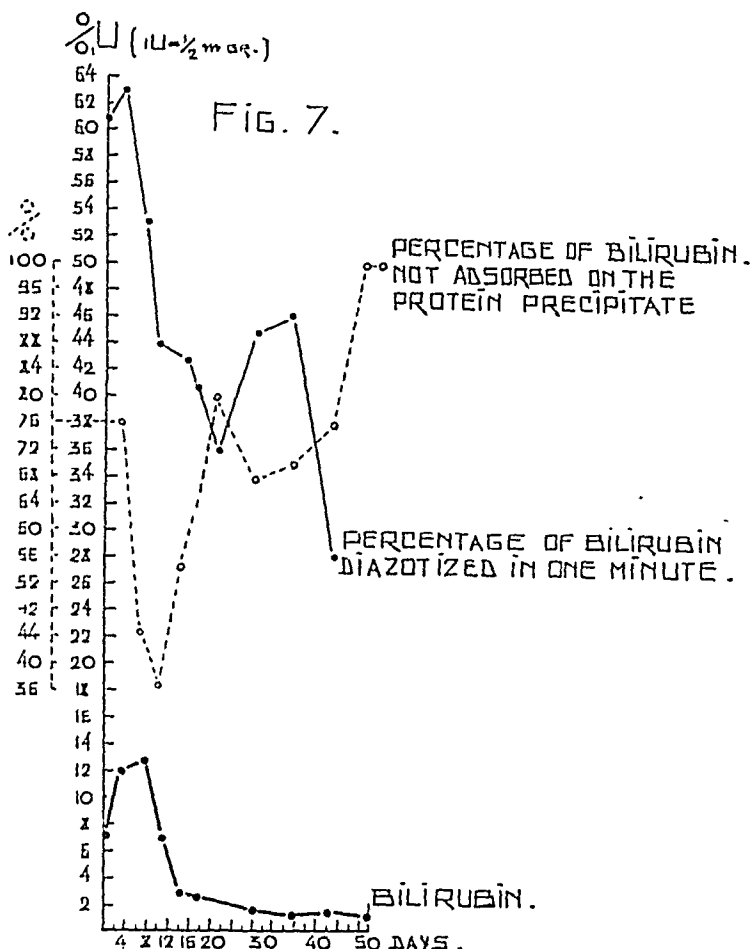


Fig. 7. Bilirubin percentage of the serum, rate of the diazo reaction and percentage of bilirubin which is not adsorbed on the albumin precipitate in a patient with icterus catarrhalis.

the rate of the diazo reaction is maximal. The second peak of the curves rendering the rate of the reaction, however, and the second part of the curves rendering the degree of adsorption are always each other's image.

Finally the rate of the diazo reaction and the bilirubinemia becomes so low, that the rate of the reaction cannot be very well determined.

In that stage the quantity of bilirubin measured with the method of Hijmans van den Bergh for indirect bilirubin, mostly corresponds with the quantity found with the method of Jendrassik and Grof (7). In that case the adsorption on the albumin precipitate is nil.

Fig. 9 shows us the result of an examination of the serum from a woman, who had or got a common duct stone after a cholecystectomy.

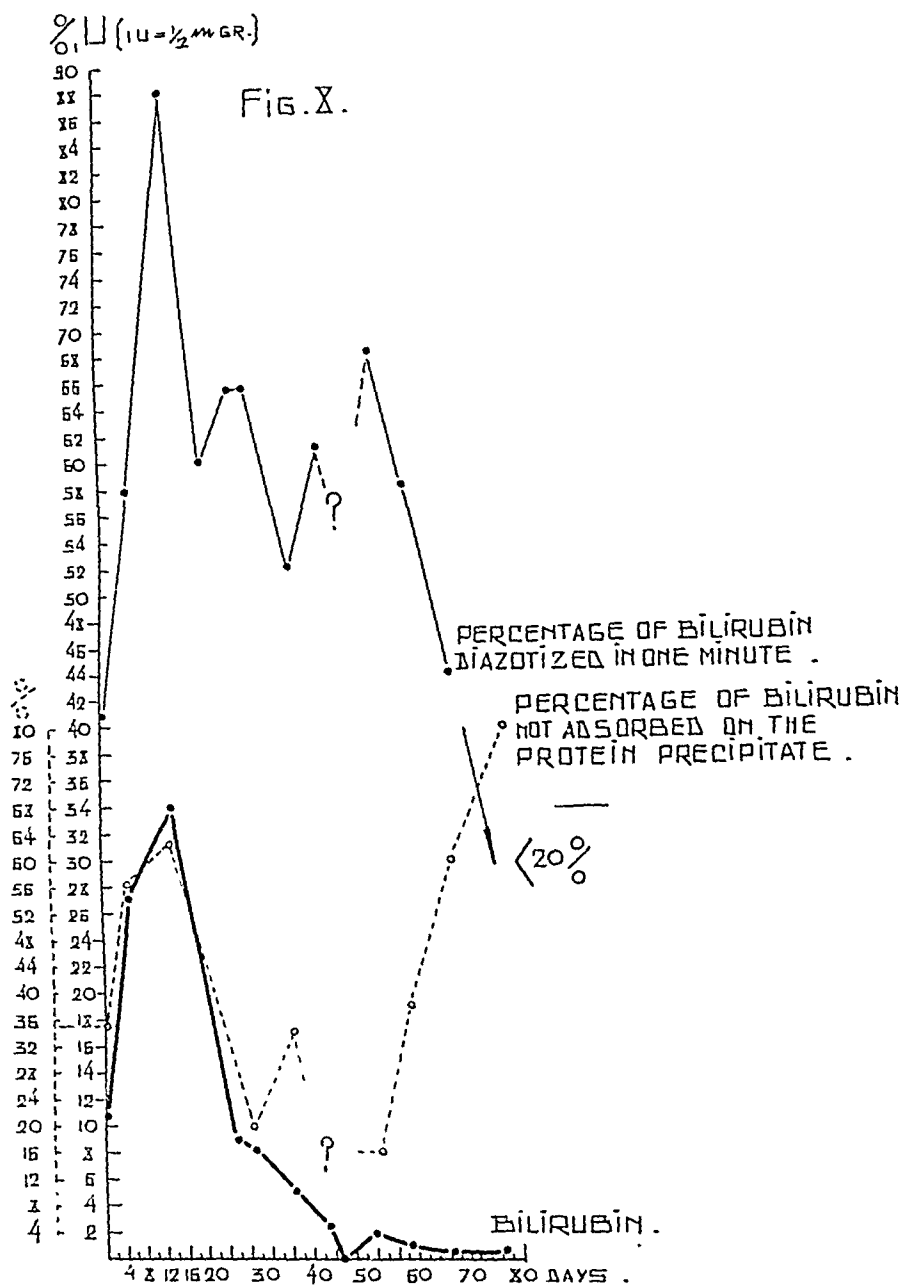


Fig. 8. Bilirubin percentage of the serum, rate of the diazo reaction and percentage of bilirubin which is not adsorbed on the albumin precipitate in a patient with icterus catarrhalis.

She was again operated upon on the day on which the first examination took place. During the operation a common duct stone was removed. After this the icterus decreased very rapidly at first, but

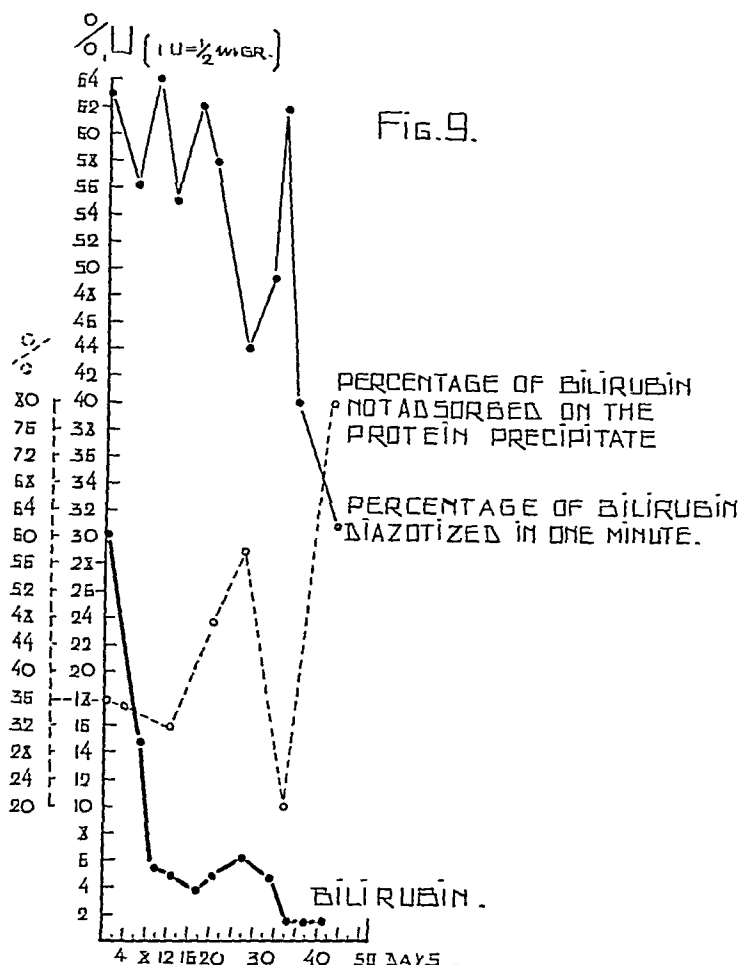


Fig. 9. Bilirubin percentage of the serum, rate of the diazo reaction and percentage of bilirubin which is not adsorbed on the albumin precipitate in a woman after the removal of a common duct stone.

slowed down afterwards and after 34 days the percentage of bilirubin of the serum had become $\frac{1}{2}$ mg. The rate of the diazo reaction, however, remained practically constant. After a decrease of the rate of the reaction we saw again a temporary increase of the rate of the reaction. Finally the woman left before the rate of the reaction had become normal. The curves rendering the rate of the reaction and the adsorption are each other's image.

Fig. 10 shows the examination of the serum from a patient with a common duct stone. Three days before the first examination of the serum, the patient had a colic. She had already had a slight icterus without any pain for three weeks. After the colic the icterus had increased. We see that the icterus decreases rapidly after the first

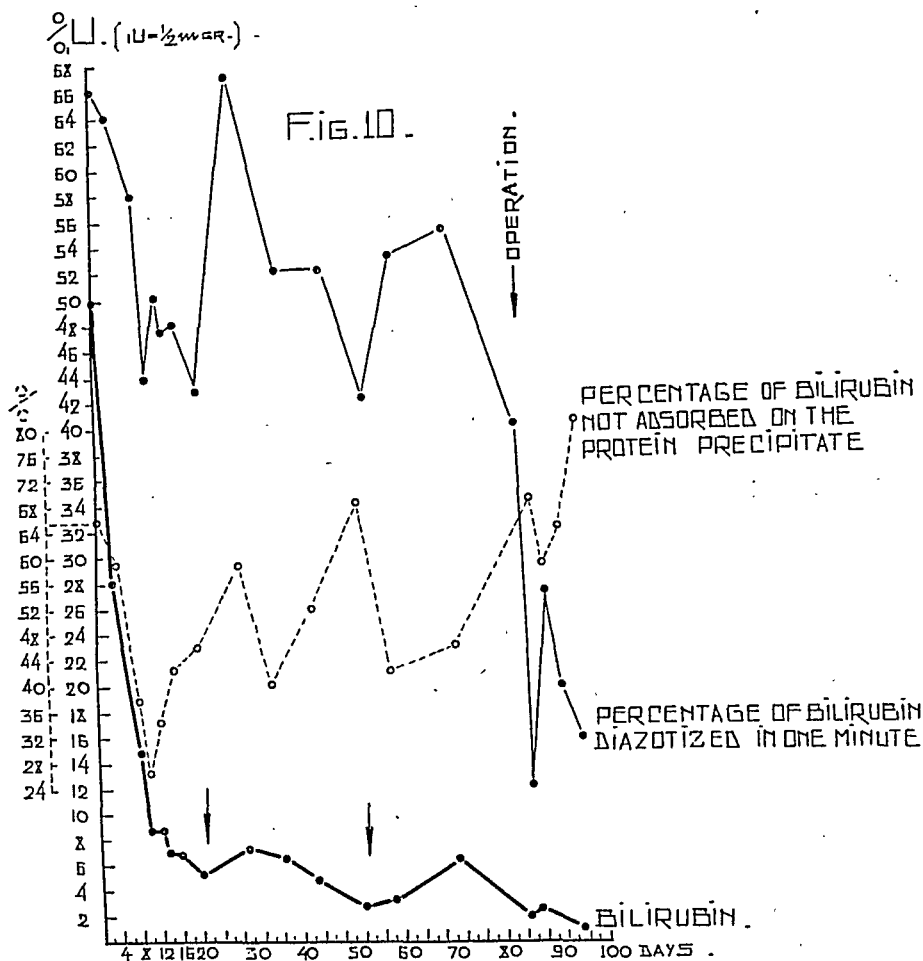


Fig. 10. Percentage of bilirubin of the serum, rate of the diazo reaction and percentage of bilirubin which is not adsorbed on the albumin precipitate in a woman with a common duct stone. The arrows indicate the days on which the patient had colics.

examination of the serum. On the 20th and on the 51st day of her stay in hospital the patient again got a colic. After these attacks the bilirubinemia and the rate of the diazo reaction increased temporarily. On the 82nd day of her stay in hospital the patient was operated upon. During the operation a common duct stone was removed. The rate of the diazo reaction decreased rapidly after the operation, but increased again a little for some days. The curves rendering the rate of the reaction and adsorption were not each other's image before and after the first colic. Before and after the second colic they were, however.

Fig. 11 shows the examination of a woman with cholelithiasis who had a colic attended with icterus 7 days before the first examination of the serum. On the 10th day the patient again got a colic.

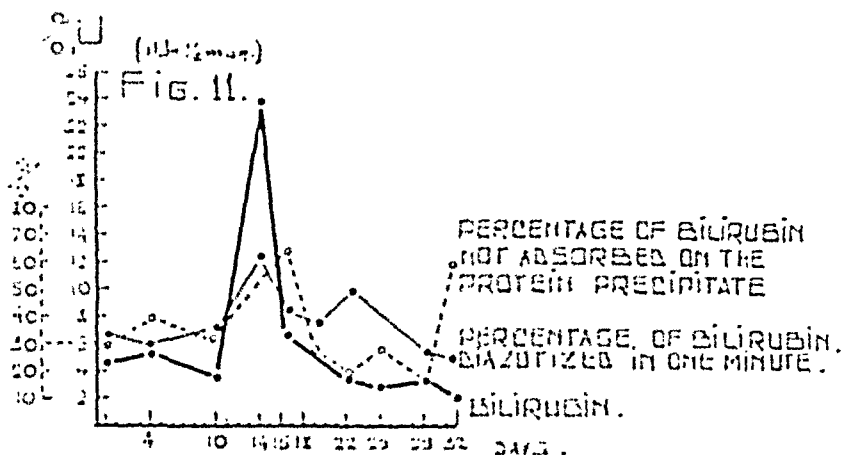


Fig. 11. Bilirubin percentage of the serum, rate of the diazo reaction and percentage of bilirubin which is not adsorbed on the albumin precipitate in a patient with cholelithiasis.

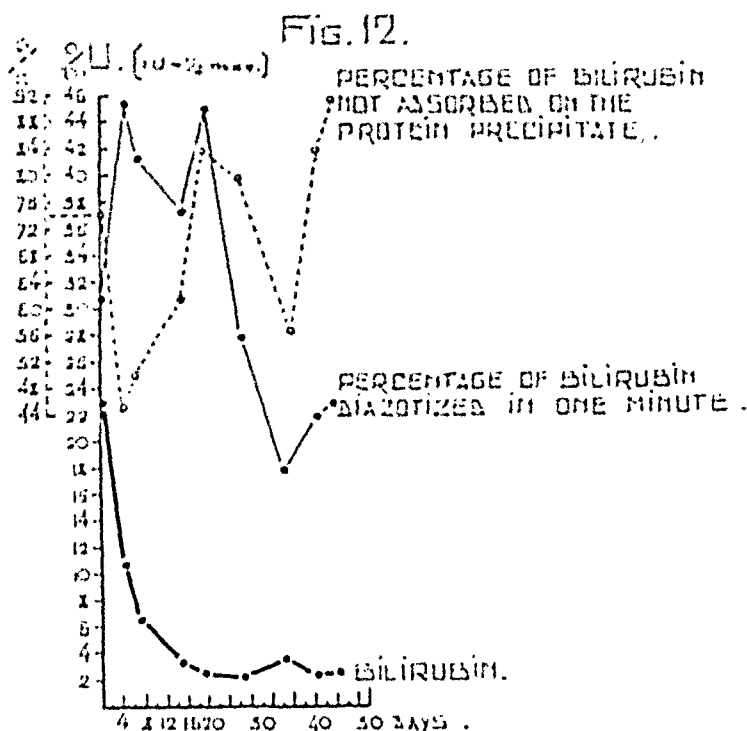


Fig. 12. Bilirubin percentage of the serum, rate of the diazo reaction and percentage of bilirubin which is not adsorbed on the albumin precipitate in a patient with heart decompensation.

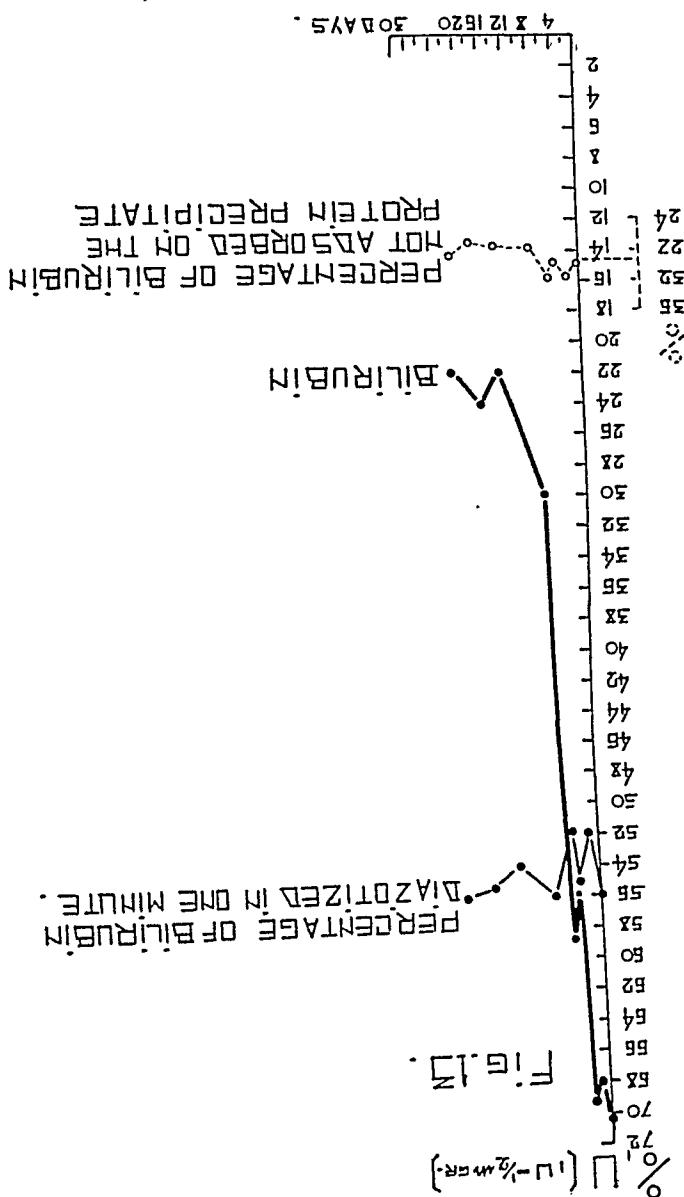


Fig. 13. Bilirubin percentage of the serum, rate of the diazo reaction and percentage of bilirubin which is not adsorbed on the albumin precipitate in a patient with a gall-bladder carcinoma after a cholecysto-gastrostomy. After that the icterus temporarily increased highly. The rate of the diazo reaction also increased greatly temporarily. The curve rendering the adsorption shows a peak, but the peak of the curve occurs later than those of the two other curves. The rate of the diazo reaction then increased again temporarily. The curve rendering the adsorption shows a fall during that time.

Fig. 12 renders the result of the examination of a patient with a serious heart decompensation with a congestion of the liver. These curves too show that the rate of the diazo reaction increases again temporarily in case of a decreasing icterus. The curves rendering the rate of the reaction and the adsorption are not each other's image by a long chalk.

Fig. 13 refers to the examination of a patient with an inoperable gall-bladder carcinoma, while a cholecysto-gastrostomy was done on the day that the serum was examined for the first time. After that the icterus decreased rapidly and the rate of the reaction and the degree of adsorption on the albumin precipitate did not change considerably.

Conclusions and Summary.

1. In case of a *rapidly changing icterus* the rate of the diazo reaction mostly increases and decreases with an increase and decrease of the icterus (*first stage*). When the icterus decreases more we see that the rate of the diazo reaction increases again temporarily (*second stage*).

2. Sometimes we see that the rate of the diazo reaction continues to increase for a little while, while the icterus is already decreasing.

3. In a patient with a gall-bladder carcinoma when a cholecysto-gastrostomy was done, the icterus did decrease rapidly but there remained a conspicuous icterus. The rate of the diazo reaction did not decrease while the icterus did decrease.

4. During the «first stage» a more rapid diazo reaction is often not attended with a stronger adsorption of bilirubin on the albumin precipitate. In the «second stage» this mostly is the case.

5. The rate of the diazo reaction and adsorption of bilirubin on the albumin precipitate are quantities which may change independently of each other. We get the impression that besides the influence on the adsorption which is exercised by the condition of the bilirubin which is present in the serum, other factors often influence the adsorption of bilirubin on the albumin precipitate. These factors are said to play an important part in the first stage.

6. In the so-called «second stage», so during the decrease of the icterus, the rate of the diazo reaction increases temporarily. I am unable to give a suitable explanation of this phenomenon.

Especially in this stage, Lups and Meyer (9) found more «not diazotizable dyes» in the serum from patients with an icterus catarrhalis. Must we look for a relation between the two phenom-

ena? Is the bilirubin in the tissues perhaps changed thus that it becomes sooner diazotizable, while at the same time products are found which do not diazotize and do these dyes and this bilirubin, which reacts more rapid, only come into the blood if the icterus decreases?

It is conceivable that oxidation products arise easily especially from quicker diazotizable bilirubin in the tissues. But why do these oxidation products only arise in the tissues and why not in th serum during the first stage, when the bilirubin is also quickly diazotizable? For the time being we can only guess.

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The Determination of the Diameter of the Red Blood Corpuscles.

By

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and GUNNAR LINDGREN.

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There are two fundamentally different methods, or rather groups of methods, used in the determination of the erythrocyte diameter, namely,

A. Halometry

B. Direct measurement with the aid of the microscope.

It is beyond the scope of this paper to return to the fundamental principles or the theoretical laws of *halometry* (*diffractometry*). The reader is referred to Cox and Ponder (1941) and Ponder (1944).

Measuring the size of small bodies (1—50 μ) with the aid of the diffraction phenomenon was made as early as 1813 by Thomas Young, who discovered and developed the principles of the method. Pijper rediscovered this principle in 1918 and used it for measuring the size of red blood cells. His apparatus, however, did not reach its final form until 1935.

Pijper's conception, given below, is not in accordance with modern theories but gives a popular explanation of the diffraction phenomenon, which appears, when a beam of light from a small aperture passes through a thin blood film. This acts as a diffraction grating when the erythrocytes are evenly spread as a single layer without overlap of cells. Around the image of the aperture there is seen a series of concentric, coloured spectrum rings. If all the cells were of exactly the same size, a distinct and well defined spectrum would be produced. The cells however, are of varying sizes and the spectrum

obtained is a resultant of the different spectras produced by the cells of different sizes. If there is a great variability in the diameters of the cells, the spectrum becomes broader and blurred. The yellow band, which is situated in the middle of the spectrum, is less influenced by anisocytosis. The halometers by Pijper, Schalm and others, constructed for measurements in the yellow band, may therefore be expected to have a greater accuracy than those where the measurements are made with the help of the red band (apparatuses by Bock, Eve and others). Cox and Ponder (1941) have described a diffractometer for use with monochromatic light, considered to give more accurate values than the older types of halometers.

Halometry offers a rapid means of computing the mean erythrocyte diameter, though the values obtained are better related to the mode than to the arithmetic mean. Another disadvantage of halometry is that it does not indicate the degree of anisocytosis (blurred spectra suggest this possibility), nor does it procure the important Price-Jones' distribution curve.

The first *direct measurements* of the erythrocyte diameter were made by van Leeuwenhoek 1674. With his primitive microscope he estimated a surprisingly correct value, 8.3μ .

Not until the 19th century were the first determinations for clinical or experimental use carried out. Manassein in Germany 1872 and Buntzen in Denmark 1879 measured the diameter of the erythrocytes by means of a scale built into the microscope. In 1883 C. Gram, using a similar method (ocular micrometry), published the results of an examination of the red blood corpuscles in man.

Another technique was introduced in 1889 by Malassez. By means of a microscope he projected the erythrocytes onto a glass plate, on which the measurement of the magnified cells could be made. Malassez has even published distribution curves resembling the later Price-Jones' curves. Price-Jones (1910 and later) has perfected the microprojection method and stands by his accurate and comprehensive clinical investigations foremost among the research workers in this field. — In 1936 Hynes and Martin reported a further improvement of Malassez' method. When measuring the cells they used a celluloid protractor into which circles of various diameters had been engraved. By projecting the cells of the preparation on to a horizontal ground glass screen they could determine the diameters by superimposing the protractor on the image obtained. The eye quickly learned to estimate with which measuring-circle a cell agreed. Their

comparative measurements showed that this method yielded as accurate results as the classical method of Price-Jones.

In both the microprojection and the ocular-micrometry technique either *wet* or *dry* preparations can be used, *e. g.* it is possible to measure the diameters of the erythrocytes, either the cells are suspended in serum (plasma) or they are fixed in a dried blood film.

Some authors advise against measurements in plasma, as the effect of anticoagulants on the size of the cells is not sufficiently known. In their own serum, however, the cells do not change appreciably in diameter during the first two hours after the sample was taken (personal observations).

Another much debated problem is if it is possible to obtain correct values using a dried blood film. Price-Jones (1920), Ponder and Millar (1924), Neale, Smallwood and Shippam (1935) found a greater cell diameter in wet preparations than in dried blood films. The difference, according to Price-Jones and to Allen and Ponder, was always almost equally great. Ponder (1944) is also of the opinion that the diameter of the cells is less in the dry film than in serum. In contrast with these authors, Collatz, however, found somewhat but not significantly lower values for the diameter of erythrocytes suspended in plasma than in the dry preparation. Finally, many investigators, some of which made the determination in plasma, others in serum, are of the opinion that the diameters determined in the wet and in the dry preparations, are the same (Ohno and Gisevius and others).

If the meanings are divergent in the above mentioned question, there is however much greater concordance in the opinion that fixing and staining the blood film do not alter the size of the cells.

Lower values (about 7 per cent.) for the diameter of the erythrocytes are obtained, when they are embedded in Canada balsam than in a dry optical system (Ohno, Eisbrich). Günther considers the decrease an optical phenomenon, brought about by the balsam. A similar diminution of the red cell diameter is obtained when oil immersion is used.

G. Larsen (personal communication; in press) has recently in an exhaustive study and by an elaborate statistical analysis been able to explain the discrepancies between the normal values of different authors. Using an ingenious photometric technique he gives a convincing statement that most of the differences observed are pure optical phenomena. He also demonstrates that the qualities of the observer's eye in some techniques may greatly influence the diameter value obtained. Finally he shows how this inconvenience can be avoided.

The investigations cited above disclose that even slight differences in the technique may markedly influence the result. By means of different combinations of these modifications, a great

Table 1.

Values of Erythrocyte Diameter found with Different Techniques.

Technique	Number of authors	Normal mean erythrocyte diameter (μ)	Range (μ)
Ocular micrometry (wet prep.)....	6	7.76	0.7
Ocular micrometry (dry prep.)....	13	7.73	1.0
Microprojection (dry prep.).....	10	7.24	0.33

many of variations in the method of measurement can be obtained. From this it will be clear how important it is that papers dealing with problems concerning the red cell diameter contain a detailed description of the method used.

The personal error may be of material interest in some techniques. As a rule all determinations in a series of blood examinations ought to be entrusted to one person only who is known to possess the necessary skill and carefulness in making accurate measurements.

Values for the mean erythrocyte diameter in healthy subjects, obtained by different authors, were collected and published by Mogensen (1938) (table 1) and by Ponder (1944). For cells measured in their own plasma Ponder considers a mean diameter of $8.5 \pm 0.5 \mu$ as most correct and for cells in dried blood films a value within the borders $7.3-7.6 \mu$.

With the microprojection method, which leaves least room for individual variation, all authors mentioned by Mogensen have measured the maximum and the minimum diameter of each cell. The agreement between the values of the different writers is better with this technique than in ocular micrometry.

In this connection Mogensen writes: »The discrepancies of the normal values obtained with the eye piece micrometer have contributed to bringing the measurement of the size of the blood cells to discredit as a clinical examination, several authors — Warburg, Lottrup, Wintrobe — concluding that the personal factors are so dominating that each investigator must determine the normal mean diameter of his own technique before he can use the method. Considering the fact that the mean diameter of normal persons varies within fairly wide limits, it is nearly impossible to fulfil this claim, and too small a series of normal persons will invariably cause faulty conclusions.»

In spite of these statements we decided to use the ocular-micrometric method, because this technique proved itself to be con-

siderably less time-consuming than microprojection and because we, with ocular micrometry, succeeded in obtaining results with a very small methodological error.

All the measurements in this paper were made by one of us (F. G.) and thus uniformity has been obtained. It is true that in measurements carried out on dried blood films we cannot be sure that the estimated size of the blood corpuscles corresponds to their size in the blood stream. But if the determinations have been performed with accuracy and with a method, strictly defined, comparable values are gained, giving clinically available information.

Method.

Wet preparations: A few drops of blood, obtained by pricking the finger, are drawn into a Pasteur pipette. After coagulation has occurred a drop of serum containing suspended erythrocytes is carefully introduced between a well cleaned¹ slide and a cover-slip. After less than half an hour the measurements are performed, the ocular ($17\times$) by drawing out the tube of the microscope being so arranged that 10 scale divisions on the eye-piece micrometer represent $10\ \mu$ on an object-micrometer. As an objective an ordinary oil immersion lens ($90\times$) is used and as immersion oil a mixture of 12 parts monobromnaphthalene and 38 parts pure liquid paraffin (Ph. S.). One hundred cells are measured, each in only one diameter parallel with the long axis of the slide.

Dry preparations: One drop of blood, obtained by pricking the finger, is placed on a well cleaned object glass. A thin smear is prepared using a cover-slip of a counting chamber or a polished edge of an ordinary slide. The film is dried immediately by waving it in the air. The smear is then directly treated in the following way:

- a) Fix in methanol for 3—5 minutes.
- b) Stain for 15 seconds in a filtered solution of 2 grammes May-Grünwald (manufactured by Grübler & Co., Leipzig) in 100 ml methanol.

¹ The glasses are cleaned in the following manner: Boil for 30 minutes in weak alkaline solution (e. g. soap), then rinse in ordinary water and finally treat with cold bichromate-sulphuric acid for several hours. Rinse again in ordinary water, transfer to 96 % ethanol and dry with linen. The slides must not be allowed to dry by themselves in the air; such glasses leave badly and irregularly stained preparations.

- c) Rinse in water pH 6.8, buffered by phosphate (Sörensen).
- d) Counterstain in Giemsa, diluted to a suitable concentration (about 2 ml concentrated reagent, prepared according to Hallman p. 252, to 100 ml distilled water), for 30 minutes.
- e) Rinse again in phosphate buffered water.
- f) Dry in the air.

The actual measurements are done in the same manner as in the earlier mentioned determinations on erythrocytes suspended in serum. Measurements are made in a central portion of the smear but in an area situated about 1 cm from its tail edge. For each adjustment 1—3 blood corpuscles, lying in the centre of the field of vision, are measured. The slide is then moved perpendicularly to its longitudinal axis, the next 1—3 cells are measured and so on until a total of 100 determinations have been made. Each erythrocyte, as mentioned before, is measured in only one diameter, parallel with the longitudinal axis of the slide.

The fixed and stained dry preparation may be kept at room temperature for weeks without any appreciable alteration of the mean diameter.

Comparison between Values from Simultaneous Determinations with »Wet» and »Dry» Technique.

In order to test whether the preparation technique had any influence on the mean diameter value or on the distribution of the cells, the following experiments were performed.

Two samples were taken simultaneously from each subject in a series of 30 persons, one sample prepared according to the »wet», one to the »dry» technique. Masel and Einhorn stated, that the mean diameter may be influenced by the order by which the preparations are made, the diameter not being the same in the first and the last drops from the puncture. By preparing one half of the »wet» samples from the first drops and the remaining half after the drop for the »dry» specimen had been taken we were able to eliminate this presumptive error. It must be mentioned, however, that in our experiments no significant difference of the mean diameter was found between the different drops. The material was divided into three groups (A, B and C), according to the mean diameter (table 2). Within each group the two averages of the mean diameter from the determinations by the »wet» and the »dry» technique were calculated.

Table 2.

*Simultaneous Determinations of Mean Red Cell Diameter by
»Wet» and »Dry» Technique.*

Series	Number of cases	Mean (μ)		Range of differences (μ)
		»Wet» technique	»Dry» technique	
A	10	8.38	8.37	— 0.19— + 0.23
B	12	7.75	7.69	— 0.22— + 0.11
C	8	7.33	7.33	— 0.15— + 0.10
Total	30	7.81	7.82	— 0.22— + 0.23

Range: Max. + and — difference within group between simultaneous determinations by »dry» and »wet» technique on samples from the same person.

Table 2 shows that the two averages within all three groups agree. The average of the 30 differences between the two determinations on each single subject was $0.024 \pm 0.023 \mu$ (calculated according to Dahlberg 1940, table 10 a). Nor could any difference in the distribution of the cells on classes with a width of 0.5μ be observed. This analysis clearly indicates that by our procedure the same mean diameter is obtained by the »wet» as by the »dry» technique.

Three cases of acholuric jaundice are not included in the comparison above between the »wet» and »dry» techniques. All four »dry» preparations from these three patients disclose a tendency to lower mean diameter values than the simultaneously made »wet» preparations:

Sample no.	1a	1b	2	3
»Wet» technique (μ)	7.23	7.20	6.84	6.93
»Dry» technique (μ)	7.04	7.02	6.77	6.89

The mean diameter of 200 cells from patient no. 1 determined in »wet» preparations is 7.210μ and in simultaneously made »dry» specimens 7.028μ . The difference 0.182μ is very probable ($t = 2.980$; $0.001 < p < 0.01$).

Variability.

Error of measure: The error when measuring a single dry preparation was established in the following way. By measuring the cells of one and the same slide 10 times, 100 cells each time, a series of 10 means (\bar{M}) was obtained:

7.78, 7.73, 7.77, 7.76, 7.78, 7.74, 7.76, 7.75, 7.78, 7.76μ

Table 3.

Probability compared values belong to same population	Significance of difference	Difference found (μ)	
		Comparison of two slides taken at random ($\sigma_d = 0.13 \mu$)	Comparison of a slide with a standard value ($\sigma_d = 0.09 \mu$)
0.05	Probable	0.26	0.18
0.01	Very probable	0.33	0.23
0.003	Significant	0.39	0.27
0.001	Highly significant	0.43	0.30

The error of measure (σ_x), calculated from this series, is $0.017 \pm 0.004 \mu$. This is a comparatively low error, being only 0.22 per cent of the average.

Error of method. As the same result is obtained whether the «wet» or the «dry» technique is used, the two averages of the mean diameter from each person can be used as a double determination, out of which the error of method (σ_e), i. e. the error involved in preparing and measuring a single slide, can be calculated from the differences between the two values. The standard deviation (σ_d) of the differences (d) between two samples from one and the same individual, taken within a short period of time, varied between 0.09 and 0.17μ in the three series tested (average 0.13μ). The error of method (σ_e) is obtained from the formula:

$$\sigma_e = \frac{1}{\sqrt{2}} \cdot \sigma_d$$

It varies between 0.06 and 0.12μ , being $0.09 \pm 0.012 \mu$ on an average. The practical implications of the magnitude of this value are seen in table 3.

The variability shows a tendency to increase with the mean diameter. Therefore, it must be observed that the values in table 3 are calculated from σ_e of normal or almost normal diameters.

The Mean Red Cell Diameter in Healthy Subjects.

In order to procure a material of normal cell diameters, 33 healthy men and 57 healthy women of all ages were investigated. Only at examination quite healthy individuals were used. Special attention was directed to the previous state of health of the subjects. All persons were excluded who complained of or had

recently complained of undue fatigue, bad taste in the mouth, anorexia and similar symptoms common in subclinic hepatic involvement. Also were rejected all cases with a previous history of diseases of the circulatory system, with anemia or other disorders of the blood, blood-forming organs and lymphoid tissue.

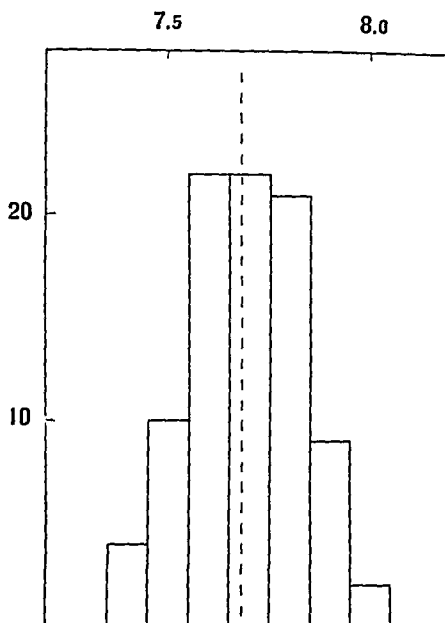


Fig. 1. Distribution of average red cell diameters in 90 healthy subjects on classes with a width of 0.1μ . 100 cells measured in each case.

Y: Number of cases. X: Mean red cell diameter.

Persons who had had any protracted period of abdominal discomfort, suggesting peptic ulcer, disorders of the liver and biliary passages or other pathological conditions of the digestive tract were also omitted. In each single case *one* sample only was prepared using the »dry» technique (see »Method»).

The standard deviation (σ) of the cell diameters in a single determination on 100 cells of a single healthy subject amounted to $0.550 \pm 0.0058 \mu$ (Range: $0.43-0.71 \mu$). This average corresponds to a coefficient of variation of 7.2 per cent. The values found agree with those given by other authors (cp. Whitby and Britton).

The average mean diameter of the whole material is to be found in table 4. There is no significant difference of the mean diameter between men and women.

Table 4.

Mean Red Cell Diameter in Healthy Subjects.

Sex	Number of cases	Mean diameter ($m \pm \epsilon_m$) (μ)	Standard deviation (σ) (μ)	Coefficient of variation (σ in per cent of mean diameter)
Male	33	7.670 ± 0.024	± 0.136	1.9
Female	57	7.693 ± 0.018	± 0.133	1.7
Total	90	7.684 ± 0.014	± 0.133	1.7

Nor is there any significant difference between men and women as regards the distribution of the cells on 0.5μ classes.

Hence it is possible to comprise the two groups into one. Figure 1 gives the distribution of the mean diameters of this material; the average of the mean diameters, $7.684 \pm 0.014 \mu$ marked by a vertical dashed line.

The standard deviation (σ) $\pm 0.133 \mu$ of the total material of 90 healthy subjects (see table 4) is comprised of two separate variabilities:

σ_i = variability between individuals;

σ_e = error of method (see p. 245).

These variabilities are related according to the formula:

$$\sigma = \sqrt{\sigma_i^2 + \sigma_e^2}$$

By inserting $\sigma = 0.133$ and $\sigma_e = \pm 0.09$ (p. 245) $\sigma_i = 0.10 \mu$ is obtained. This variability between individuals of 0.10μ is only 1.3 per cent of the mean and thus very small.

The mean diameters of healthy subjects, when examined according to the principles stated above (p. 242) will vary between the limits given in table 5. For this practical application $\sigma = 0.133 \mu$ has been used, this error including both the methodological error and the variability between individuals.

The standard deviation (σ) is more or less independent of the number of cases in a material. The range, however, is determined by the number of cases, a larger number showing a larger range etc. Tippett has calculated the relation between the range and the number of cases and given the numerical values of the range expressed in fractions of σ . According to Tippett a material of 90 individuals has a range of 4.94σ , or $\pm 2.47 \sigma$. From this value and $\sigma = 0.133 \mu$ we calculate the range 0.328μ or $7.35-8.01 \mu$.

Table 5.

*Range of Mean Diameter in Healthy Subjects.*Mean: 7.68 μ σ : 0.133 μ

Standard deviation		Calculated range	Per cent of group within range	Per cent of group above upper limit
σ	μ			
2 σ	0.27	7.11 — 7.95	95.5	2.27
2.5 σ	0.33	7.35 — 8.01	98.8	0.62
3 σ	0.40	7.28 — 8.08	99.7	0.13
3.3 σ	0.44	7.24 — 8.12	99.9	0.05

The actual values found varied between 7.38 and 7.99 μ : a close agreement with expectation.

The investigation has been supported by grants from the Medical Research Council of Sweden and from the Foundation »Therese and Johan Anderssons Minne», for which we here express our sincere gratitude.

Summary.

The mean diameter of the red blood cells may be determined either by means of diffractometric methods or by direct measurement. The last technique certainly is more time-consuming than the first one but gives more correct information as to the real average diameter. The direct methods have another advantage: by delivering a Price-Jones' curve they give a rather exact statement of the degree of anisocytosis. A brief introductory review is given of different opinions and investigations of the two techniques.

The next section of the paper gives a rather detailed description of measuring cells suspended in their own serum or fixed in dried and stained blood films (eye-piece micrometer technique).

Several authors have stated that greater cell diameter values are obtained in »wet» preparations than in dried blood films. In simultaneous determinations with »wet» and with »dry» technique we did not find any difference, however (table 2).

The error (σ) when measuring a single dry preparation is low, being only 0.2 per cent of the average. The error of method (σ_0) was calculated according to the formula $\sigma_0 \times \sqrt{2} = \sigma_d$, where

σ_d was the standard deviation of the differences between two samples taken as nearly as possible simultaneously from the same subject. σ_e varied between 0.06 and 0.12 μ , being $0.09 \pm 0.012 \mu$ on an average.

The normal mean red cell diameter was determined in a material of 90 healthy subjects. The mean standard deviation (σ) of the cell diameters in a single determination on 100 cells of a single healthy subject was $0.550 \pm 0.0058 \mu$, *i. e.* a coefficient of variation of 7.2 per cent. The average mean diameter was $7.684 \pm 0.014 \mu$ (table 4). No difference between men and women was observed. The $\pm 2.5 \sigma$ range of the mean diameter in healthy subjects was 7.35–8.01 μ (table 5).

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Necrotizing Generalized Arteritis Due to the Use of Sulfonamide Drugs.

By

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Symptomatology.

Within a short time two cases of anuria after the use of sulfonamides were observed, which seemed to be due to pathological changes of the renal parenchyma rather than to an obstruction of the urinary tract by sulfonamide crystals.

Both patients recovered after a long time during which the specific gravity of the urine remained constantly low.

Two further cases of renal damage after the use of sulfonamides will now be described.

First case: A 62-year-old man, known to be suffering from hypertension and impaired renal function, contracted purulent parotitis. The patient received sulfathiazole in a dosage of 6 g in two days and was then admitted to hospital because of a very low urinary output, 300 ml per 24 hours. In the urine albumin and erythrocytes were found. With forced fluids the urine production increased but the urea content of the blood increased to 3.9 g/l and the patient died after an illness of 12 days duration. The parotitis, due to staphylococcus, had quickly cleared up with penicillin. The diagnosis was damage to the kidney due to sulfathiazole. The post-mortem findings were as follows: Both kidneys had a peculiar patchy appearance and on microscopical examination vascular changes were noticed; the pathologist judged these to be allergic, but was unable to decide whether they were due to the chemotherapeutic used or to the infection.

Second case: A young woman became acutely ill with abdominal pain and diarrhoea and was treated with sulfathiazole. She was ad-

mitted to hospital with a diagnosis of appendicitis (?). On admittance she was an extremely sick woman. The temperature was only slightly elevated. Because the stools were bloody a tentative diagnosis of gastro-entero-colitis was made and because of vomiting oral sulfonamide therapy was discontinued and replaced by injection of sulfathiazole. The temperature rose and pending the outcome of the bacteriological examination the woman was started on penicillin. She became, however, steadily worse, complaining of pain in the upper abdomen; an ice bag was applied over the gall-bladder region. Exudate in the right pleural cavity without signs of subphrenic abscess. On the 6th day of hospitalisation severe polyneuritis suddenly developed and rapidly became worse. The right radial nerve was most seriously involved, the right hand could not be lifted. Dr. S. Duursma, consulting neurologist, noticed absence of tendon reflexes, the sensibility was disturbed as well as the vibratory sensibility. The fundi were normal, also the cerebro-spinal fluid. Polyarthrits developed and a transient painful reddened swelling of the right upper arm. Sulfathiazole was discontinued immediately at the first signs of polyneuritis and penicillin and vitamin B₁ were continued. On the 10th day of hospitalisation the urine secretion was diminished to 200 ml/24 hours and oedema developed. Blood pressure rose from 120/80 to 160/120. In the urine albumin and erythrocytes. The urea content of the blood at first increased to 2.6 g/l and rose further to 3.9 g/l. The specific gravity of the urine did not exceed 1015. A transient parotitis cleared up again. The temperature dropped a little. Paralytic ileus developed and death ensued. Extensive bacteriological and serological examination of blood and faeces was entirely negative. These negative findings and the widespread localisation in various organs suggested a diagnosis of periarteritis nodosa, although no nodules were felt. Autopsy showed the same patchy kidneys as described in the first case, also diffuse enteritis. Microscopic examination showed inflammation in the small arteries of almost every organ.

Similar observations were found in the literature.

In 1942 Rich (1) described lesions which he termed periarteritis nodosa (but which actually were more like generalized arteritis) in patients, who had received massive doses of serum and in whom serum sickness had preceded death. All these patients had also received sulfonamides. We are of opinion, that it is uncertain whether these lesions were caused by the serum or by the sulfonamide compounds. There was also one patient, who received sulfonamides but no serum and had the same microscopic lesions. Rich describes his cases as necrosis of the walls of the smaller arteries with infiltration of the wall and the surrounding connective tissue. A few cases also had interstitial myocarditis and local necroses were observed in some. These lesions had been described previously by authors studying experimental anaphylaxis in animals.

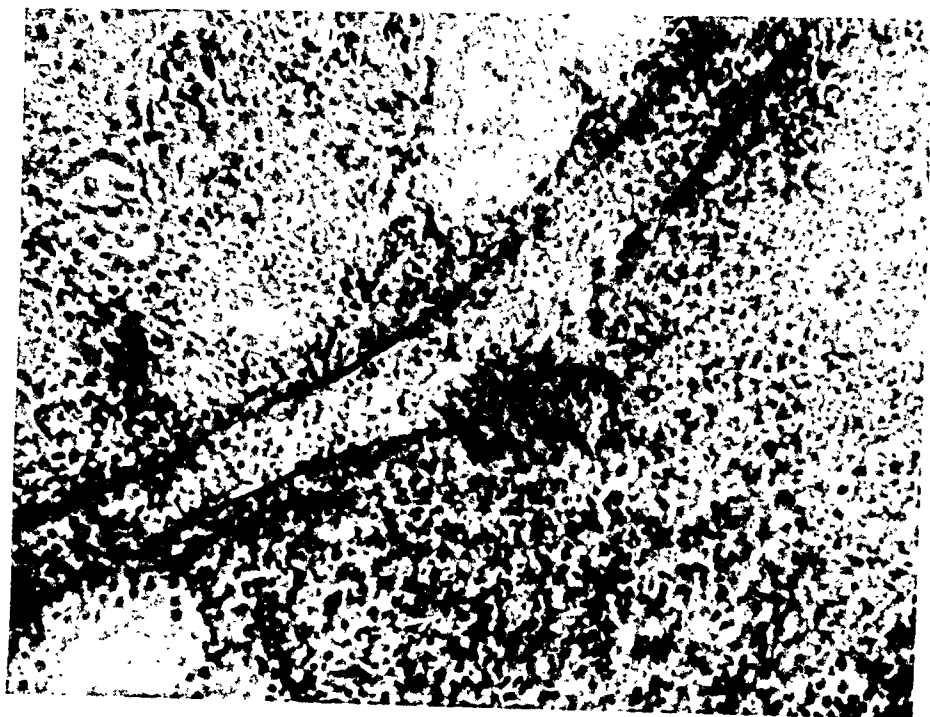


Fig. 1. Case 2 kidney.

Lengthwise section of an artery. In the lumen many leucocytes, the wall is infiltrated, as well as the perivascular connective tissue. In the centre a side-artery, the wall is necrotic and infiltrated. $\times 150$.

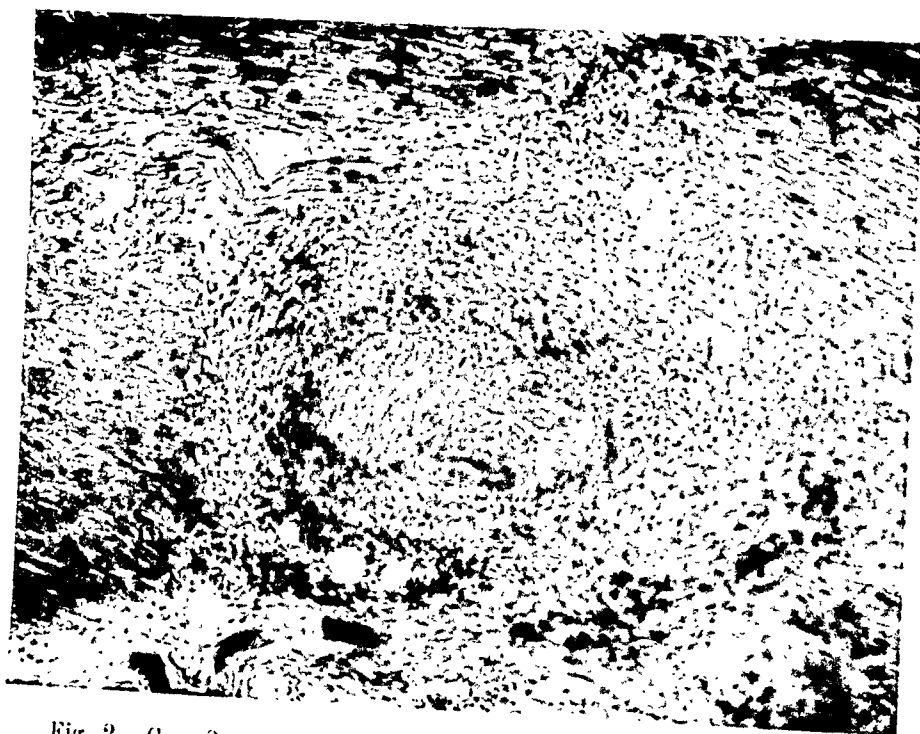


Fig. 2. Case 2 myocardium. Dense infiltration around an artery. $\times 95$.

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Fig. 3. Case 3 kidney.

Dividing artery. The vessel wall is very much swollen and is fibrinoid-necrotic. The surrounding tissue is infiltrated. $\times 170$.



Fig. 4. Case 1 kidney.

Different cross-sections of a tortuous artery. The intima is thickened (arteriosclerosis). The vessel wall is darkly stained in many places due to necrosis. In the adjacent tissue infiltration. $\times 170$.

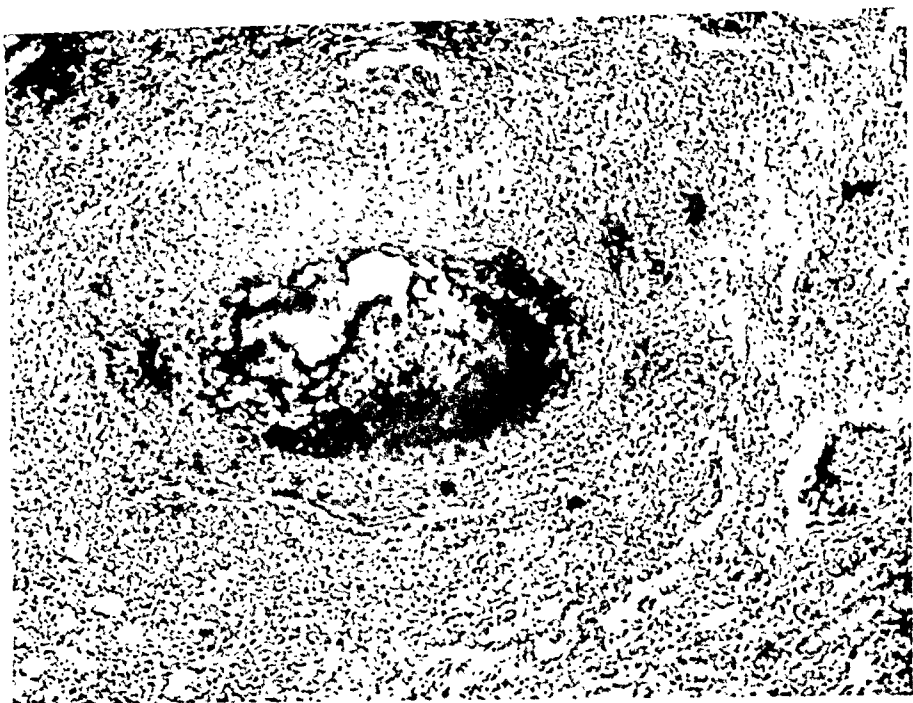


Fig. 5. Case 4 kidney.

A fairly large artery partly filled with thrombosis. The wall is infiltrated and necrotic in various places. $\times 75$.

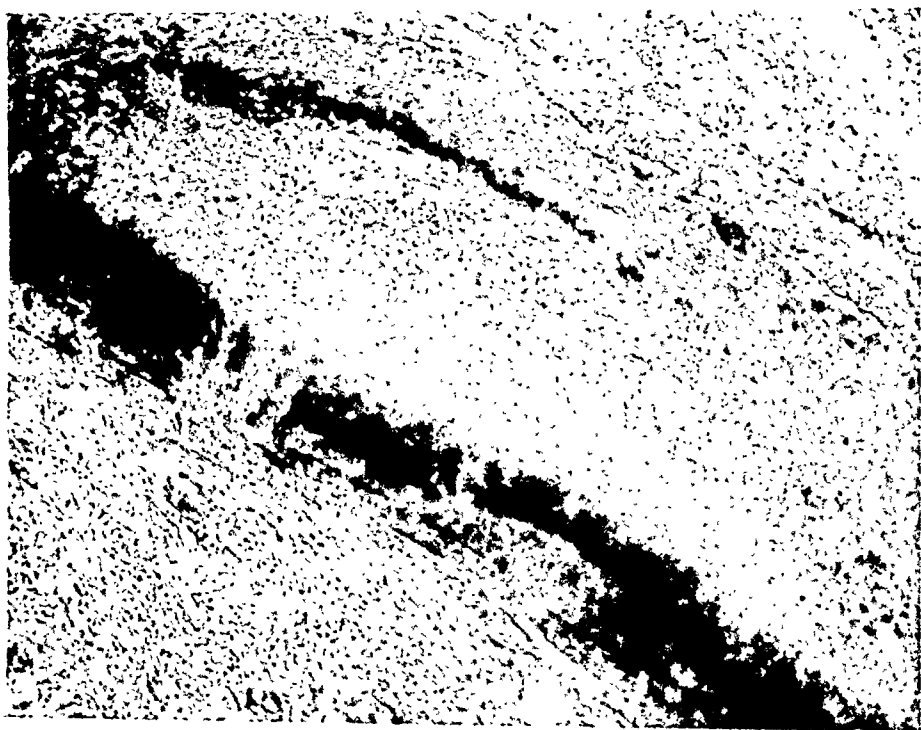


Fig. 6. Case 5 kidney.

The wall of a large artery is almost entirely necrotic. In the lumen thrombosis, the wall is impregnated with blood. $\times 75$.

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Rich and Gregory (2) also observed these lesions in their experiments on rabbits with induced serum sickness.

Lederer and Rosenblatt (3) noticed slightly different lesions after sulfonamide drugs, which they described as foci of necrosis surrounded by infiltration. Furthermore they found degenerative changes in the kidneys and interstitial myocarditis. Similar observations were made by Merkel and Crawford (4).

There is also an important article on this subject by Black-Shaffer (5) published in 1945 reviewing the literature and adding 5 cases observed by the author himself. These authors also draw attention to the fact, that the infiltrations were mostly situated in the perivascular tissue. The sulfonamide medication was the only factor common to these cases.

In 1946 there follows the description of a case by Lichtenstein and Fox (6) in which sulfathiazole had been applied to the skin for wound-treatment.

More, Gardner, Mc. Millan and Duff (7) studied 2,000 post mortem findings between 1940 and 1944; 375 patients had received sulfonamides. In 22 cases lesions were found which might have been caused by sulfonamides. In 7 cases death could only have been due to sulfonamide drugs and the lesions were again the same as those described above, which will be further illustrated below. A survey by French (8) of 76 autopsies showed, that all known sulfonamide drugs, even sulfoguanidine and other poorly soluble sulfonamides, may cause these lesions. The amount administered varied from 8—340 g, the period of administration varied from 2 days to a few weeks. Half of these cases had shown typical symptoms of hypersensitivity to sulfonamides such as dermatitis and other skin eruptions, conjunctivitis, icterus, aplastic anaemia, fever, eosinophilia, hemolytic anaemia and leucopenia.

There are still a few further arguments in favour of the hypothesis, that those vascular lesions described above are related to sulfonamide-hypersensitivity.

a. Arthus' phenomenon, produced by protein hypersensitivity is also a fibrinoid necrotizing inflammation of the walls of the small arteries with cellular exudate in the surrounding tissue.

b. Shaffer, Lentz and Mc. Guire (9) were able to transmit sulfonamide-anaphylaxis to normal persons by means of the Prausnitz reaction. They injected the serum of patients, who were hypersensitive, to one of the sulfonamide compounds intracutaneously into healthy persons. If a diluted solution of the same sulfonamide

was injected in the same place the next day, the skin reacted with the formation of a weal, whereas the untreated skin did not react in this way.

c. Sulfonamide may on occasion acquire the properties of an antigen, by binding itself to serum protein (Schönholzer and Davis).

Two more cases of generalized necrotizing arteritis were found among the autopsy-reports of the pathological institute at Groningen. These patients had also received sulfonamides and their cases were similar to those mentioned above, so that it is very probable, that sulfonamide was the cause of death.

Third case: A woman fell ill with symptoms of severe diarrhoea and fever. She was given sulfathiazole. Bacteriological and serological findings during life were negative. This patient developed polyneuritis, also with dropping hand and nephritis with uraemia and death ensued. Post mortem paratyphus B bacilli were cultured from the spleen.

Fourth case: Patient suffering from sinusitis and asthmatic bronchitis with fever; sulfathiazole was given. The frontal sinus was opened, pus appeared, but the temperature remained high. The patient became definitely worse and sulfathiazole was discontinued in favour of sulfapyridine. The patient became uraemic and changes in the urine suggested the presence of nephritis. Anaemia developed and the patient received vitamin B injections (neuritis?). Death ensued.

In both cases microscopic findings were the same.

By chance we discovered a fifth case, described to us by a colleague who had diagnosed it as periarteritis nodosa during life, while the autopsy had revealed necrotizing lesions of the vessels. We thank Dr. Klein for informing us of this case and are obliged to Dr. Mansens for presenting us with the organs.

Fifth case: Sulfathiazole was prescribed for a woman by her medical attendant on account of signs of pneumonia. The temperature dropped at first to normal, but rose again on the next day and became 0.1° to 0.2° higher every day. Negative findings but the patient was very ill with high fever. Changes in the urine, rising blood pressure, oliguria of 100 ml/24 hours. Although the urine secretion increases it appeared, that the kidneys were unable to concentrate. The patient became uraemic and anaemic and died. In the first weeks an eosinophilia of 6 % was found in the blood. The tendon reflexes were diminished and there was conjunctivitis. A rapidly fatal course was observed in the next case, who had already been treated with sulfathiazole during scarlet fever two years previously.

Sixth case: A young man, 19 years old, complained of sore throat, the physician found redness of the fauces and prescribed sulfathiazole

because the patient had had rheumatic polyarthrits, which had damaged the mitral valve. The temperature was 40° C. and remained unchanged in spite of the chemotherapy. The patient became gradually worse. On the third day of illness the flow of urine stopped, the patient became delirious and shock developed. The consulting neurologist Dr. Duursma diagnosed polyneuritis. All reflexes were absent the abdominal reflexes excepted. Cerebro-spinal fluid normal. There was a scarlatiniform rash without any further signs of this disease (the patient had already had scarlet fever). The sclerae were injected. In the scanty urine obtained per catheter albumin and erythrocytes were found. The urea content of the blood was 1.2 g/l. The clinical picture was obscured by shock. Blood transfusions were without effect. The urine production continued very low and the patient died on the fourth day. Bacteriological examination of the blood was negative, the spleen was sterile. On microscopic examination inflammatory changes were most prominent especially in capillary regions. The very rapid course was surprising. The patient had received only 11 g of sulfathiazole, but it was certain, that the same drug had been administered before.

In the following case the diagnosis of periarteritis was made because of the clinical picture, probably with the same underlying cause. This patient will probably recover, although he has nephritis.

Seventh case: A 41-year-old man consulted us because of attacks of asthma during the past year. He also had sinusitis frontalis. Physical examination showed only bronchitis. The sputum contained many eosinophile cells and eosinophilia in the blood was 22 %. Potassium iodide and bromide and ephedrine were prescribed and the patient was instructed to take his temperature at home. The temperature was elevated and sulfathiazole was prescribed by the patients' own medical attendant, in all 52 g were given. After 4 weeks we saw the patient again. The temperature was still high, appreciably fluctuating. There was no dyspnea, but the patient expectorated. The patient was admitted to hospital. The first 4 days he received 6 g sulfathiazole daily and, because the temperature remains high, penicillin as well. The sputum cultures were invariably sterile. Blood cultures were also sterile. X rays of the lungs showed small patches of increased density over the entire left lung. Eosinophilia 57 %, after one week 62 %. Sedimentation rate 30 mm, Kahn test positive, M. B. R. + citochol test +. Typhoid fever, Bang's disease and Ratbite fever negative. The temperature was distinctly fluctuating. The marked eosinophilia, changes in the lung, asthma, fever and non-specific serologic reactions were somewhat reminiscent of the recently described »tropical fever». But the patient had never visited the tropics and outside the tropics this disease has never been seen up till now. Salvarsan was tried, as it was known to give excellent results in tropical fever. The temperature dropped gradually, but the pulse-

Table I.

Cases	1	2	3	4	5	6	7
oliguria	300	200	—	—	100	200	—
specific gravity	1005-1013	1012-1015	?	1006-1014	1006-1012	1017-1019	1007-1016
albumin-uria	(+)	$\frac{1}{2}$ ‰	3 ‰	+	(+)	(+)	(+)
erythrocytes	very numerous	very numerous	numerous	—	numerous	numerous	40-50
blood pressure	180/100	120/80→ 160/120	185/100	120/80	120/70→ 160/100	<80	120/70→ 160/100
blood-urea g/l	2→3.9	2.6→3.6	0.5→1.2	2.6	2.9→3.75	1.2	1.2

rate remained high and the general condition became rapidly worse. The man could not sleep, vomited and became emaciated. Eosinophilia decreased slowly: 43 %, 39 %, finally 15 %. Polyneuritis developed and the man became mentally disturbed. As he had had 8 salvarsan-injections the neurological symptoms were thought to be related to the salvarsan, but this was ruled out by the neurologist Dr. Duursma. Coordination was disturbed, there was ataxia, the reflexes were diminished or absent, sensibility distinctly impaired in distal regions. Spinal fluid normal. Anæmia developed, hemoglobin decreased from 90 % to 54 %, the urea in the blood was 1.2 g/l. In the urine little albumin, 30 to 50 erythrocytes per field. Specific gravity not over 1016. Blood pressure was still normal, but the man showed signs of dehydration, hypodermoclyses improved his condition. Then transfusions were given. Gradual improvement. The temperature, already almost normal, became entirely so, the pulse rate also returned to normal, though slowly. The mental condition cleared up, the patient started to eat again, the vomiting stopped. The urea content of the blood was dropped on a low-protein diet, (0.5 g/kg body weight) but increased again, when more protein was given. The specific gravity of the urine did not exceed 1016 even on a low liquid-intake. Repeated urea clearance tests gave a result of 50-60 %. Abnormal findings in the urine persisted and the blood pressure rose to 160/100. The electrocardiogram (showing a negative T₂ and T₃ wave at first) became normal again. The changes in the lung found on the X-ray also disappeared. The man put on weight, 39-46.1 kg. Serological tests for syphilis became negative. Eosinophilia only 5 %. The polyneuritis also showed a tendency towards improvement.

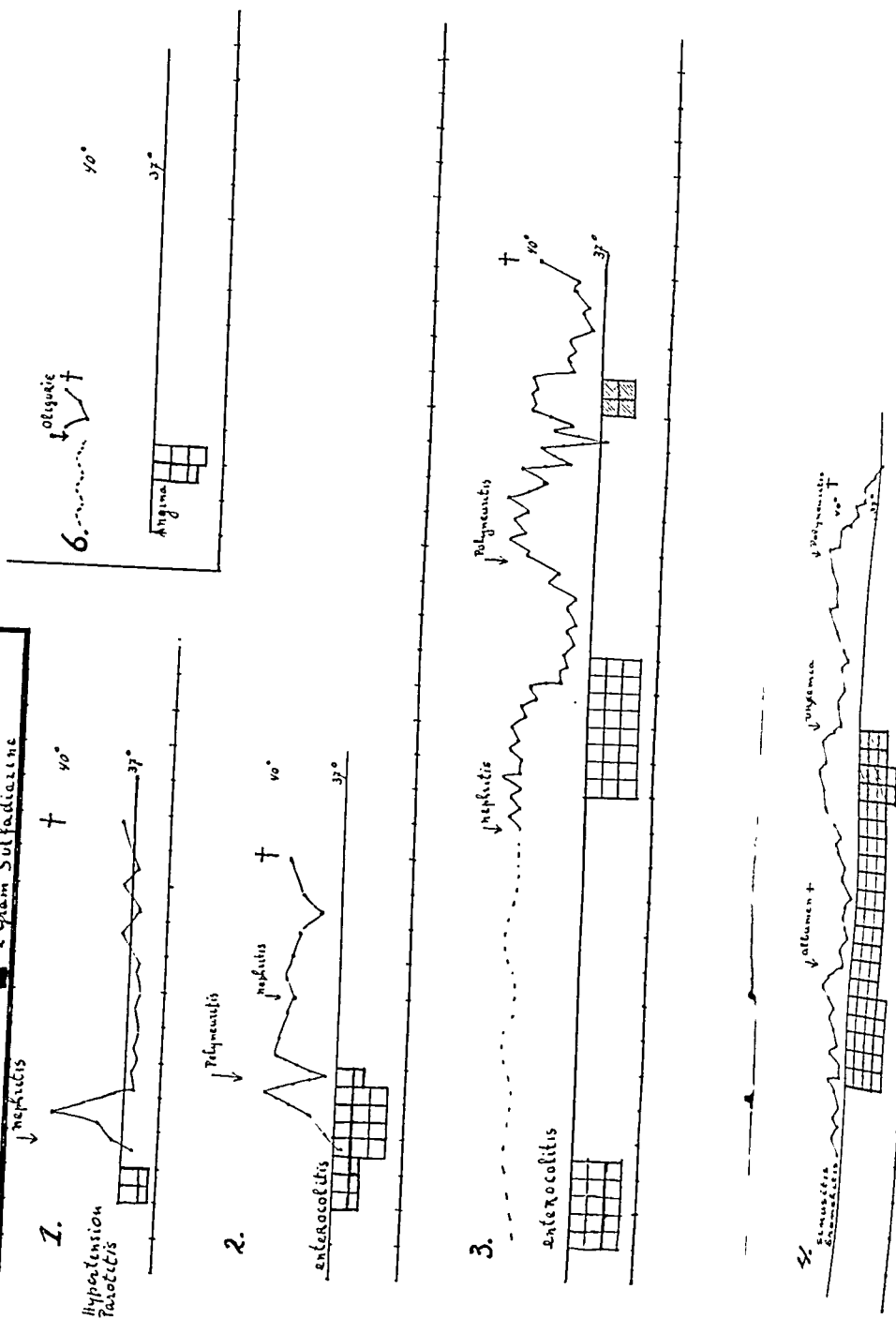
Marked eosinophilia and undulating fever have been met in periarteritis (nodosa). The localisation in the abdomen, nerves,

Table II.

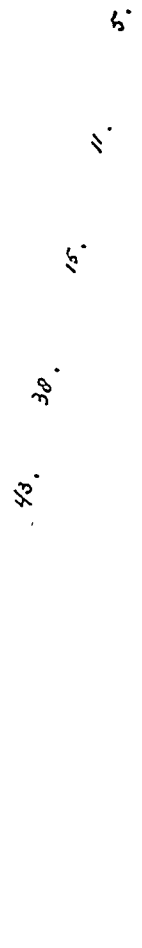
Patients	1	2	3	4	5	6	7
Age	♂ 62	♀ 38	♀ 42	♂ 58	♀ 49	♂ 19	♂ 41
Previous sulfonamide treatment	?	?	+	?	—	sulfathiazole two years before	?
Allergic symptoms	—	—	—	conjunctivitis after K J	injection of sclerae	injection sclerae Rash	asthma
Hemoglobin	→ 40 %	70 %	4 transfusions	106→60 %	→ 35 %	120 % (shock)	90→54 %
Leucocytes	—	23,000 22 % stabs	19,000 16 % stabs	21,500 15 % Eos.	7,200 6 % Eos.	25,400 34 % stabs	25,000 62 % Eos.
Hemorrhagic symptoms (thrombocytes normal)	mouth nose	skin	—	—	nose	nose	—
Restlessness	+	+	+	+	—	+	+
Nephritis	+	+	+	+	+	+	+
Polyn neuritis	—	+	+	+	+	+	+
Other localisations		abdomen pleura joints parotis arm				heart	lungs heart cerebrum abdomen
Bacteriologic examinations	Parotis staphylococ.	Blood — Spleen — Feces —	Spleen paratyphus		Blood —	Blood — Spleen —	Blood — Sputum —
Autopsy	nerotizing arteritis kidneys myocarditis	generalized nerotizing arteritis myocarditis	generalized nerotizing arteritis	generalized nerotizing arteritis myocarditis	nerotizing arteritis kidneys myocarditis	generalized vasculitis myocarditis hepatitis	
Remarks	Wa. R. —	Cong. Syphilis Wa. R. —			Carcinoma ventriculi.	Scarlatina + Rheumatic fever previously	Syphilis Reactions aspecific + later —

TABLE III

Temperature	
-----	at home
—	in hospital
□	2 gram Sulfathiazole
▨	4 gram Sulfapyridine
■	2 gram Sulfadiazine



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heart, lungs and kidneys was in favour of this diagnosis. Microscopic confirmation would have been useful, but excision of a lymph gland, a small artery and some muscle tissue was only possible after the symptoms had practically cleared up. Nothing abnormal was found then. Skin tests and the Prausnitz reaction were then also negative. (An identical lung picture was described by Svanberg, *Acta Radiologica* 1945, 307—312 in arteritis nodosa.) We think it probable, that this man suffered from arteritis identical to that of our other patients.

Omitting case 1 and 6 because the illness was very short, we find a real clinical entity in the other cases. In the first place we are struck by the fact that so many organs showed pathological changes (table I). All cases had nephritis with an insidious onset (table II). Red blood cells in the urine and a trace of albumin. Gradually the blood pressure rose and the patient became uræmic. The kidneys were unable to concentrate sufficiently, whereas there was still some dilution, especially in the rapidly fatal cases there was severe oliguria. Sometimes this was the first alarming symptom. Besides the nephritis, polyneuritis was an important part of the clinical picture: diminished reflexes, paralyses and disturbance of sensibility. Gradually increasing anemia. A characteristic feature was the marked restlessness of the patients. Undulating fever and eosinophilia occurred in one half of the cases. Skin eruptions should be looked for in cases of this kind.

Table III gives details of the course of the disease in our patients to facilitate comparison.

Pathology.

In all cases where autopsy was performed identical changes were found. These were: necrotizing inflammation of the blood vessels, especially of the arteries. The walls of these vessels contained infiltrating cells, mononuclear as well as polynuclear cells, showed fibrinoid swelling and partial necrosis. The elastic fibres had vanished. The lumen of some of the vessels contained many polynuclear leucocytes, a few vessels showed thrombosis. The tissue around the vessels was infiltrated by polynuclear cells. The changes in the vessels covered a great extent. This definitely marks the process as different from that in periarthritis nodosa, which is limited to a small area of the arteries, causing small nodes to develop. In our cases these nodes were absent. In cases 1, 2, and 3

the small arteries were especially involved. In the kidneys these were the lobular arteries. Because of these changes in the vessels the renal parenchyma showed localised degeneration, the epithelium of the tubules in these areas being markedly swollen. This explains the patchy aspect of the kidneys.

In cases 2 and 3 necrotizing arteritis was found in many organs (fig. 1, 2 and 3). In the kidneys, liver, heart, muscle, intestinal mucosa and peripheral nerves several small arteries shared in this process. The clinical symptoms of nephritis and polyneuritis may well be explained by this fact. Undoubtedly death in these cases was due to these lesions.

In case 1 arteritis existed only in the kidneys (fig. 4). This patient had been suffering for a considerable time from hypertension and arteriosclerosis and had previously been uræmic. The arteries in the kidneys were distinctly sclerotic. The changes due to arteritis were less marked here than in cases 2 and 3, but since they had developed in kidneys, which already functioned very poorly, symptoms of renal insufficiency set in immediately, ending in fatal uremia.

In case 4 the calibre of the arteries involved in the kidneys was larger than in the preceding cases (fig. 5). The damage was very serious here. The same changes were found in heart, liver, lungs and spleen. Macroscopically the kidneys were patchy and had a mottled aspect, in the lungs were thrombi, which had caused hemorrhagic infarction.

In case 5 the arteries involved were still larger (fig. 6). In the kidneys there were the lobar arteries. They could be recognised macroscopically as grey-white stripes 2—3 mm wide. The wall of these vessels was entirely necrotic and thrombi had developed. In several places this had caused anæmic necrosis in the renal tissue.

These 5 cases showed similar changes in the arteries of many organs, only one case had lesions only in the kidneys. Most of these cases also showed signs of slight interstitial myocarditis. From experimental studies it is known that allergic conditions may lead to generalized necrotizing arteritis and interstitial myocarditis (Metz (10), Klinge (11), Rintelen (12), Rich & Gregory (2)). The question arises what may cause this allergic state. Neither the course of the disease, nor the clinical symptoms made it likely that allergy was due to infection, although the patients were originally suffering from an infectious process which had been the reason for sulfonamide treatment.

The infective agent differed widely in the various cases. Case 1 staphylococcus-parotitis; cases 2 and 3 enterocolitis (salmonella was cultured from the spleen post mortem in case 3); case 4: purulent sinusitis with bronchiectasis; case 5: pneumonia; case 6: angina. There was no evidence of sepsis in any of these cases; the infection had practically subsided when symptoms of necrotizing arteritis developed.

It is therefore not probable that the allergic state, underlying the necrotizing inflammation of the vessels, was due to the original infections.

Clinically it appeared that the symptoms were related to the administration of sulfonamides and later it became apparent that they were due to arteritis.

Moreover the lesions observed in our cases were similar to those described in the American literature as allergic inflammation of the vessels due to sulfonamides. In view of these considerations we are of opinion that the lesions which caused the death of our patients and which should be described as necrotizing generalized arteritis were due to sulfonamides.

The noxious action of the sulfonamides may be explained in two ways:

a. Sulfonamides, after conjugation with protein, act as antigen which leads to allergic reactions.

b. Sulfonamides cause disintegration of micro-organisms, in this way liberating substances which act as antigens.

The experiments of Verlinde and Zeldenrust (13) render it likely that the second explanation is the right one. But in view of the great variety of infections in our cases and the results of Shaffer, Lents and McGuire (9), who demonstrated a positive reaction of Prausnitz-Küstner with sulfonamides, it seem more likely, that sulfonamides may have antigenic properties.

In case 6 the small arteries and the arterioles were especially involved. Necrosis of the vessel-wall was less marked, infiltrations contained chiefly polynuclear leucocytes. In different areas the inflammatory process was localised around the capillaries. These lesions were found in heart, lungs and liver. In the kidneys glomerulitis and periglomerulitis were noted. There was also polyserositis. These findings differed somewhat from those in the other patients, but they were in agreement with the clinical symptoms. The illness ran a peracute and fulminating course and the patient died in severe shock. He had been treated with sulfathiazole two

years previously. Probably this explains his violent reaction and it is correct to suppose that the patient had already been sensitized to sulfathiazole. In this case also, we think that sulfonamide was responsible for the death of the patient.

These observations point to a distressing fact. That sulfonamides could become dangerous was already known. Generally speaking the internist is less eager to administer these drugs than are the general practitioner and other specialists. The dangers are comparable to those of an operation. It is not to be predicted how a patient will react to operation, and no more is it possible to predict whether he will tolerate sulfonamide medication. We know of but one way of eliminating the dangers of sulfonamides, that is not to use them. In the U. S. A. the desirability of using the sulfonamides in hospitals only has already been expressed. We must use sulfonamides and we are not in a position to limit the administration of the drug to the hospitals only. But it is of great importance that every doctor should realise more and more that sulfonamides are drugs which most decidedly should not be prescribed as one prescribes aspirin. They are not household remedies.

We should like to suggest the following rules:

1. *Clinical Use:*

Penicillin is the drug of choice. In all cases where penicillin is useful no sulfonamides should be given. The use of sulfonamides is only justified in those cases where bacteriological examination has shown the presence of serious disease against which penicillin is of no avail. The indication for use of a dangerous drug should be just as carefully decided as the indication for a serious operation. It is obvious that it is impossible for practical reasons to hospitalize every case of pneumonia, but a patient with pneumonia admitted to hospital should receive penicillin because it is less dangerous.

2. *Use in general practice:*

Never give sulfonamides for fever only. The popular name of »fever-tablets» for sulfonamides clearly explains the bad habits which exist here.

If sulfonamides are absolutely necessary, they should be given in large doses during a short time. If in a case of pneumonia treated with the correct dosage of sulfonamides the temperature has not

dropped within 48 or at the latest within 72 hours, the drug may as well be stopped. The patient should then be taken to hospital. The use of sulfonamides over a long period in small doses should be avoided: it does no good and may sensitize the organism.

3. The drug should be immediately stopped if the temperature rises again after an initial lowering.

4. The volume of the urine should be measured every day and should not be less than 1,500 ml.

5. Allergic symptoms should be looked for: skin eruptions, conjunctivitis, eosinophilia, polyneuritis, changes in the urine, polyarthrititis, asthma, restlessness; if there are present use of the drug should be discontinued immediately.

Summary.

Seven patients are described who suffered from generalized necrotizing arteritis. In six of them this illness had a fatal course and autopsy could be performed. The authors regard this arteritis as allergic and as caused by preceding treatment with sulfonamides. They warn against these drugs and give some therapeutical rules, intended to prevent this dangerous complication.

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A Simple Quantitative Calcium-Formolgel Reaction, and its Connection with the Euglobulin and Gammaglobulin Content of Serum.

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The estimation of the protein content of blood is relatively seldom carried out, although the symptomatic, diagnostic and prognostic importance of the composition of the blood protein is not inferior to that of the morphological blood picture. This finds its obvious explanation in the fact that the methods for the determination of the content of the various protein fractions are partly complicated and time-consuming (chemical methods) while others demand the use of very costly apparatus (electrophoresis and ultracentrifuge methods).

Moreover it is seen that the knowledge of the changes in the serum protein content values obtained by one single determination is often not of great value, but by following the course of the changes they become of much more importance.

In the course of the past few years there have been important advances made in the possibility of more easily obtaining information about the protein »spectrum». The dependence of some older reactions, including that of Takata-Jezler and its modification, the Mancke-Sommer reaction, and of the formol-gel reaction of Gaté and Papacostas, from the serum protein spectrum has been further established by comparison with the protein spectra obtained by chemical and electrophoretic methods. (Gutman and Wise (1), de Vries (2), Verschure (6).)

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Newer reactions which have been developed include the Hayem titration of Gros, the sublimate titration of Stolte, the cadmium reaction of Wunderly and Wuhrmann, the thymol turbidity test of MacLagan and the cephalin cholesterol flocculation test of Hanger.

It has been shown that all these reactions become positive in case of raised serum globulin content, and principally the raised content of gammaglobulin, together with which a decrease in the albumin has a further stimulating influence, varying with the different reactions.

In assessing the clinical value of these and similar reactions to determine changes in the serum protein content in pathological conditions the following particulars are of importance:

1. From the reaction it must be decided as far as possible which abnormality in the protein spectrum is responsible for a positive reaction.

2. The technique and the preparation of the reagents must be as simple as possible, so that the method can be used routinely without objection.

3. The reaction must be virtually independent of the environment, such as changes in the temperature, etc.

4. The result of the reaction must be capable of being read clearly and objectively, so that comparable results can be obtained by different investigators.

5. The reaction must be sensitive and give quantitative results *i. e.* it must allow of accurate graduation of the serum protein changes which it indicates, from the slightest to the greatest degrees seen in pathological states.

This last aim is of great importance, for the reaction which fulfils these requirements enables us to follow the changes concerned quantitatively during the course of an illness. It is not fulfilling this aim when some reactions give a false semblance of specificity for a definite disease.

This »specificity» is only based on a fairly high threshold value, which the reaction possesses for the causal changes in the serum protein content, although essentially these changes occur often and to a less extent in numerous other diseases. This has led to a great many investigations of doubtful value, concerning, for instance, the Takata-Jezler reaction in various liver disturbances and its whether or not being specific in certain liver diseases.

All the above mentioned protein reactions are deficient in one

or more of the five points laid down. As the basis of an extensive investigation with simple methods, of the changes in the serum protein spectrum during the course of several dermatological and venereal conditions, I used a number of these reactions.

The deficiencies already mentioned occasioned the development of a modified formol-gel reaction, which, after having been prepared on empirical grounds, was submitted to a comparative investigation with the protein spectra obtained by precipitation by means of solutions of sodium sulphate of various concentrations, following the methods of Howe and Majoor¹ and finally with the results obtained along electrophoretic lines.² It thus appeared that a reaction had been found which fulfilled all five conditions very satisfactorily.

The method was derived from the very simple formol-gel reaction of Gaté and Papacostas, in which 1 ml of serum and 2 drops of neutral 40 % formalin are mixed, and which reaction is considered to be positive if a gel has formed after 24 hours, of such a consistency that it does not run out of the inverted tube.

The investigators who have studied this reaction closely are in agreement in so far as they all consider that an increase in the globulin content of the serum is responsible for the positive result. There is, however, some difference of opinion as to whether the total serum globulin content, or only the increase in the euglobulin, is the determinant factor. (Gutman and Wise (1), de Vries (2), Biguria and Foster (3), Strauss and Kaunitz (4) and Bing (5).)

By comparative studies using electrophoresis Verschure (6) obtained strong indication that the formol-gel reaction becomes positive when the gammaglobulin content exceeds a value of 26 gm/litre. The great majority of investigators, on the other hand, are of the opinion that a decrease in the albumin has no, or at most only slight, influence on the reaction. The formol-gel reaction should thus be a measure of the euglobulin (gammaglobulin) content of a specimen of serum.

¹ The determinations of protein spectra by the methods of Howe and of Majoor were carried out by Miss Dasia and Miss v. d. Wal, analysts in the laboratory of the Medical Clinic of the Binnengasthuis at Amsterdam, and were part of the routine work of that laboratory. I am greatly indebted to the head of this department, Professor J. G. G. Borst for his readiness to assist, his interest and his invaluable advice.

² The electrophoretic analysis were carried out in the apparatus of the State Veterinary Research Institute, Amsterdam, by Dr. L. W. Janssen, to whom I am also grateful for his valuable criticism. I am very indebted also to the director, Dr. H. S. Frenkel, for the warm hospitality of that Institute.

If we limit ourselves, however, to a reading of the reaction after 24 hours, and consider only complete gel formation, only scanty information is provided by the reaction. It divides the sera under investigation into only two types, those with a euglobulin (gammaglobulin) content above a definite level and those with a content below that level.

Although the formol-gel reaction has, on the one hand, attractive possibilities as far as its great simplicity, objectiveness, the predominant influence of only one protein fraction, and its almost negligible sensitivity to temperature changes are concerned, it has, on the other hand, two important shortcomings. These are, namely, its high threshold value, and the fact that its results are unsatisfactory for further quantitative estimations. In positive cases this can be overcome by noting the *time* in which gel formation occurs or carrying it out on diluted serum, in negative cases by estimating the change of viscosity in the absence of complete gel formation.

Both shortcomings appear to have been overcome. Firstly, it was possible to lower the threshold value of the reaction at choice. This was done by the addition of calcium chloride solution along with the formalin. The concentration and quantity of the calcium chloride solution could be so determined empirically that in «normal cases» after 24 hours there was just no gel formed. After that the reaction was further modified to give quantitative results by carrying it out on a series of serum dilutions, diluted progressively with physiological saline, when it could be determined up to which dilution gel formation occurred.

The resulting reaction, henceforth to be called the calcium formol-gel reaction (Ca.F.G.R.) is set up as follows:

Tube. Nr.	I	II	III	IV	V	VI	etc.
Serum ml.	1.0	0.95	0.90	0.85	0.80	0.75	etc.
NaCl 0.9% ml.	—	0.05	0.10	0.15	0.20	0.25	etc.
Formalin 40% } CaCl ₂ , 12% } equal parts ml. ...	0.10	0.10	0.10	0.10	0.10	0.10	etc.

Method of Carrying Out the Ca.F.G.R.

A series of tubes is used, 8 cm in length and 1.5 cm, internal diameter. Reagents and serum are added according to the table, by means of pipettes calibrated to 1/100 ml. After shaking the

tubes and bringing all droplets remaining on the walls down to the rest of the fluid, the tubes are closed with rubber corks. They are then allowed to stand for 24 hours at room temperature, when the result is determined by inverting the tubes one by one in order to find up to which dilution a gel has been formed, of such consistency that the contents of the tube do not run down.

The boundary between the last positive and the subsequent tube is in most cases sharply demarcated. Occasionally, however, there is, following the last positive tube, one tube in which the contents run very slowly down after inversion and gentle movement. This tube, which does not fulfil the criteria required in order to be considered as positive, but in which the contents is not completely fluid, is given the values $\frac{1}{4}$, $\frac{1}{2}$ or $\frac{3}{4}$.

Preparations of Reagents.

Calcium chloride solution. 12 g crystallised calcium chlorid ($\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$) are dissolved in a measuring flask to 100 ml. The strength of the solution is confirmed by determining the specific gravity. This should be 1.048. The solution should be neutral to bromthymol blue.

Formalin. 4N sodium hydroxide is added to commercial formalin solution until it is neutral to bromthymol blue. The formol concentration is then measured by the method of Romyn. If necessary it is then diluted until the concentration is 37.0 g/100 g, *i. e.* 40.1 g/100 ml. The reaction and concentration are checked from time to time. The neutral solution may be stored for several months.

A mixture of equal parts of these two reagents is made immediately before the reaction is carried out, and should not be done in advance.

The reaction is adjusted that, as appeared evident from a considerable number of normal cases, no gel formation occurs in the first tube. The changes found in the pathological conditions reported here showed a variation between 1 (undiluted) and 15 (dilution of 30/100). Very seldom are more than 10 tubes positive.

The results obtained were very constant. A great number of reactions were carried out in duplicate, and agreeing closely with each other, it was permissible to give a result, for instance, of $4\frac{1}{2}$ (dilution 83/100).

The reaction was in all cases read after 24 hours. This period of time was chosen from analogy with the standard formol-gel reaction and because this time is generally the most convenient on practical grounds. This period should be adhered to, as the reaction is by then not complete. If the tubes are left to stand for a further 24 hours the positive result is found to occur about two tubes higher. It is not necessary, however, to adhere strictly to the 24 hour period, for the process continues so slowly after one day that it matters little if the result is read an hour too early or too late.

It is necessary, to have the tubes corked, for otherwise gel formation may occur in a thin surface layer in one or two tubes next to the one with the last positive reaction as a result of evaporation, making it difficult to assess the true result.

The reaction is only slightly sensitive to changes in the temperature. If however it is allowed to proceed for 24 hours in an incubator at 37°C gel formation occurs two dilutions further along than if it is carried out at room temperature. If carried out in a refrigerator a stronger reaction is also found, *i. e.* one tube higher than at room temperature. Both extremely high and low temperatures give too high values. It is advisable, therefore, on very warm days to place the tubes in a cool place.

The blood should be taken off with the minimum of congestion of the part. After clotting has taken place, the clot is freed from the inside of the tube and the blood kept for 24 hours at room temperature. The serum is then poured off and centrifuged. If the serum must be kept for a few days it should be placed in a refrigerator, this has no effect on the reaction.

The objection that much serum is required for this reaction may be met in three ways. Firstly, one may use a less finely graduated serum dilution range. The results obtained by so doing are naturally less accurate quantitatively. To prevent confusion if a different serum dilution range is used, it is advisable to state not only the number of tubes in which gel formation has occurred, but also the serum dilution in the tube in which the last positive result is obtained.

Secondly, one may use smaller tubes and half the quantities of serum and reagents. Although this appears satisfactory there is obviously a greater chance of making appreciable errors in the pipetting of these smaller amounts of fluid, while narrower tubes

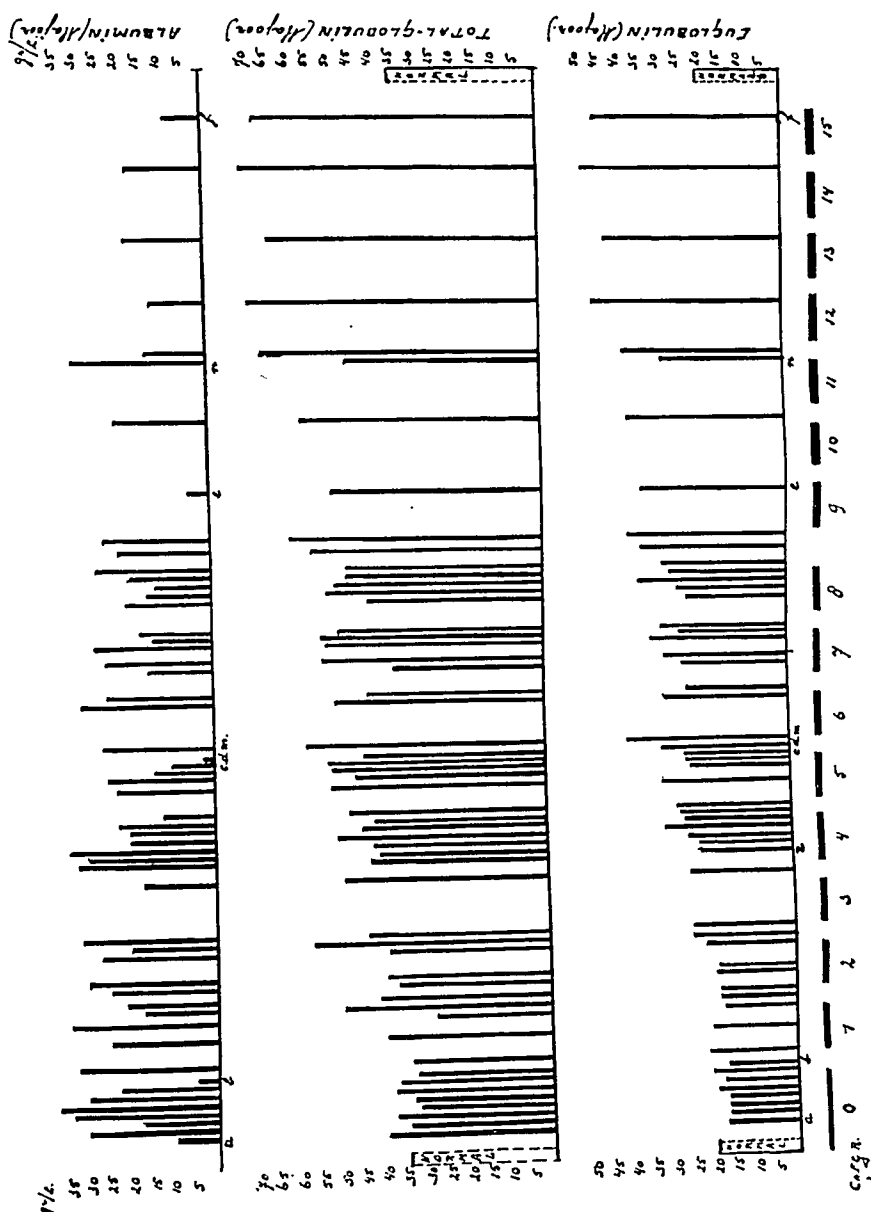
have more tendency to prevent an incomplete gel from running down on inversion of the tube, and in order to obtain results comparable with those from the larger tubes, the smaller tubes should be shaken slightly.

Finally, one may use less serum if, as soon as sufficient serum has separated, a standard-formol-gel reaction is set up using only 1 ml. the rest of the serum being kept over till the following day. By comparison of a large number of sera it has been found that sera with a positive standard-formol-gel reaction show gel formation in the Ca.F.G.R. in 7 or more tubes (dilution 70, 100 or higher) and sera with a negative standard-formol-gel reaction show gel formation in the Ca.F.G.R. in at most 7 tubes (at most up to dilution 70/100). By first setting up a standard-formol-gel reaction one is able to determine in advance in which zone one should set up the Ca.F.G.R. and the reaction can be restricted to the first seven tubes, while in others it may be begun at the seventh tube.

The albumin and globulin content of the sera of 51 patients was determined using the method of Howe (end-concentration of sodium sulphate of 215 g/litre) and the protein spectra were determined by Majoer's method (7) (end-concentrations of sodium sulphate of 270 and 184 g/litre.) The patients whose sera were investigated came largely from the Medical Clinic, and partly from the Department for Skin and Venereal Diseases. By this means material was obtained from a wide range of diseases, with the result that the changes encountered in the serum protein spectrum were of a very varied nature. The protein spectra of these sera were studied in correlation with the changes found in the Ca.F.G.R. To this purpose graphs were drawn, from which the relationship between the Ca.F.G.R. and the various fractions could be assessed.

From these graphs it was evident that an undeniable relationship existed between the result of the Ca.F.G.R. of a serum and the total globulin content (determined by Howe's method), but, at the same time, that a pure quantitative parallelism between this fraction and the intensity of the Ca.F.G.R. was not present. (This graph is not illustrated here.) This could also be deduced by studying the graphs of the total globulin content obtained by using Majoer's method. In Graph I this correlation can readily be seen. The correlation was here less marked than on comparison with the total globulin as determined by Howe's method.

It is observed also that the irregularities in these graphs cannot



Graph 1. In this graph is shown the protein spectrum of 54 sera as found using Majoor's method, with the corresponding values for the Ca.F.G.R. The abscissa represents the number of tubes showing gel formation in the Ca.F.G.R. The protein fractions are given as vertical lines, the albumin (above), total globulin, i. e. pseudoglobulin and euglobulin (centre), and euglobulin (below), all in g/litre. The normal values for the euglobulin and the total globulin are the averages of a number of observations of Majoor (7) and are shown to the right and the left of the graph.

be explained by attributing to changes in the albumin content the power of exerting any definite influence on the reaction.

Because Majoor (7, 8) has made it sound very acceptable that by means of the concentration of sodium sulphate of 270 g/litre advocated by him, a fuller separation of the albumin and globulin is brought about than by the concentration given by Howe, the probability became obvious out of the above conclusions from the two graphs, that it is not the total globulin content of the serum which determines the strength of the Ca.F.G.R. but the content of one or more of the globulin sub-fractions.

Graph I also illustrates the correlation found with the euglobulin content as determined by Majoor's method. The sera with a Ca. F.G.R. of one or two tubes already show an average euglobulin content, higher than do the sera with a negative Ca.F.G.R. After the second tube of the Ca.F.G.R. the euglobulin content of all sera is clearly greater than 20.8 g/litre, which value is considered by Majoor as a provisional upper limit of normal, and the progression as the Ca.F.G.R. becomes stronger is more regular than in either of the above-mentioned graphs. Here also, there are some irregularities which cannot be explained by taking into consideration the albumin content of the sera concerned. If one looks at sera a, b, c, d, e and f, for instance, which sera are characterised by a marked hypo-albuminemia, then it is nowhere found that this diminution in the albumin has made these sera come further to the right in the series than would be expected on consideration of their euglobulin content. We find thus that there is no indication that the low albumin figure has any significant »advancing» influence on the Ca.F.G.R.

In connection with the predominantly good correlation between the euglobulin content (Majoor's method) and the result of the Ca.F.G.R. shown by Graph I, it should be noted that the protein spectrum determinations have formed part of the routine investigations in the Medical Clinic. They were not performed with the more than usual care otherwise employed in a scientific investigation. Improbable results were not repeated, in order to exclude errors due to mistakes. This should be borne in mind when further on the results obtained by electrophoresis and by Majoor's method are compared.

In the cases of two sera, m and n, which do not fit correctly into Graph I and which appear very considerably out of place, the electrophoretic protein spectrum is known (Nr. VII and XIV

Tab-

Normal values	Total Protein (Kjeldahl) g/l 65—80	Electrophoresis: relative values				Electro-absolute	
		alb. % 57.1— 66.9	α glob. % 4.8— 10.1	β glob. % 10.3— 13.2	γ glob. % 17.4— 23.4	alb. g/l 38.9— 47.9	α glob. g/l 4.7— 5.8
I. Weber-Christian's disease	66.5	50.0	10.1	10.4	29.5	33.3	6.7
II. Erythrodermia psoriatica	76.0	53.0	8.7	10.0	28.3	40.0	6.6
III. Xanthoma tuber. mult.	75.0	45.3	6.2	25.9	22.6	34.0	4.7
IV. Atopic eczema....	71.0	48.9	6.4	12.1	32.6	34.7	4.5
V. Pemphigus vulgaris	65.0	41.3	12.4	13.9	32.4	26.8	8.1
VI. Ac. diss. lupus erythematosus	65.0	34.2	12.6	10.3	42.9	22.2	8.3
VII. Gonococcal arthritis	85.0	41.7	11.5	13.1	33.7	35.4	8.8
VIII. Secondary syphilis	82.0	46.7	5.1	12.4	35.8	38.3	4.2
IX. Tertiary syphilis..	80.0	41.2	9.4	14.1	35.3	33.0	7.5
X. Lepromatous leprosy	64.0	18.4	25.0	17.2	39.4	11.8	16.0
XI. Lymphopathia Venereum	74.0	40.2	8.3	17.6	33.9	29.7	6.1
XII. Pemphigus vulgaris	79.0	33.0	13.5	16.4	37.1	26.0	10.7
XIII. Ac. diss. lupus erythematosus.....	87.0	42.7	6.2	9.5	41.6	37.1	5.4
XIV. Ac. diss. lupus erythematosus.....	79.0	36.8	5.2	9.0	49.0	29.1	4.1
XV. Ac. diss. lupus erythematosus	81.0	28.3	8.4	7.9	55.4	22.9	6.8
XVI. Secondary syphilis	83.0	30.4	5.6	13.0	51.0	25.2	4.6
Column:	1	2	3	4	5	6	7

Column 1. Consists of the total protein contents (Kjeldahl). The normal values are taken from de Vries (2) and Gutman (10).

Columns 2—5. Give the relative values of the electrophoretic fractions, expressed as a percentage of the total. The normal values are taken from Westermann (9) as found by him in a number of normals under the same test conditions and using the same apparatus.

Columns 6—9. The absolute values of the electrophoretic fractions, calculated from the relative values and the total protein (Kjeldahl).

The figures offered as normal values are calculated from the average relative values of Westermann and the values given as normal for the total protein.

Column 10. The result of the Ca.F.G.R., recorded as the number of tubes in which gel formation occurred, the reaction being carried out as described on page 268.

1c 1.

phoresis: values		Ca.F.G.R. Number of tubes	Protein Spectrum (Majoor)			Protein Spectrum (Howe)		Total glob.		Albumin/gammaglobulin ratio (Electrophoresis)	Mancke-Sommer Reac- tion: Number of tubes	Blood Sedimentation Ra- te in one hour (Westergren)
β glob. g/l 7.7— 9.5	γ glob. g/l 13.6— 18.6		Eu- glob. g/l 20.2— 20.8	ps. glob. g/l 10.3— 16.4	alb. g/l 39.5— 47.5	alb. g/l 50.4— 60.4	glob. g/l 16.4— 26.3	Maj. g/l ± 34.5	Elect. g/l 26—32			
6.9	19.6	0	16.0	18.9	31.6	42.3	24.2	34.9	33.2	1.67	0	24
7.6	21.1	2 $\frac{1}{2}$	25.0	18.1	32.4	45.5	30.0	43.1	35.6	1.87	1	6
19.4	17.0	4	22.8	19.6	32.6	44.5	30.5	42.4	41.1	2.00	2	3
8.6	23.1	4	22.3	17.7	31.0	44.0	27.0	40.0	36.2	1.50	2	3
9.0	21.1	4 $\frac{1}{2}$	26.3	17.7	20.5	33.5	31.0	44.0	38.2	1.27	6	68
6.7	27.9	4 $\frac{1}{2}$	27.0	14.5	23.5	32.0	33.0	41.5	42.8	0.80	7	34
11.1	28.6	5 $\frac{1}{2}$	40.0	18.0	27.0	35.5	49.5	58.0	49.5	1.20	2	69
10.2	29.4	6 $\frac{1}{2}$	31.0	19.2	31.8	41.0	41.0	50.2	43.8	1.30	5	75
11.3	28.1	7 $\frac{1}{2}$	34.1	18.0	28.0	38.1	42.0	52.4	47.2	1.16	7	97
11.0	25.2	8	36.8	13.2	14.0	21.9	42.1	50.0	52.2	0.47	7	129
13.0	25.1	8	30.3	16.8	27.2	38.1	35.9	47.1	44.2	1.18	7	49
13.0	29.3	8 $\frac{1}{2}$	36.0	21.0	22.0	39.0	40.0	57.0	53.0	0.89	7	40
8.3	36.3	8 $\frac{1}{2}$	39.0	21.8	26.2	42.7	44.3	60.8	49.9	1.03	5	54
7.2	38.7	11 $\frac{1}{2}$	30.5	16.0	32.7	42.0	37.2	46.5	49.9	0.75	7	95
6.1	44.9	11 $\frac{1}{2}$	40.0	26.0	15.0	30.0	51.0	66.0	58.1	0.51	8	125
10.8	42.3	12	47.5	22.0	13.5	27.7	55.3	69.5	57.7	0.59	7	106
8	9	10	11	12	13	14	15	16	17	18	19	20

Columns 11—13. Protein spectrum by the Majoor method. (Euglobulin placed first.) The normal values are those at present given by Majoor. (7, 8.)

Columns 14—15. The albumin and globulin content (Howe's method). Normal values taken from de Vries. (2.)

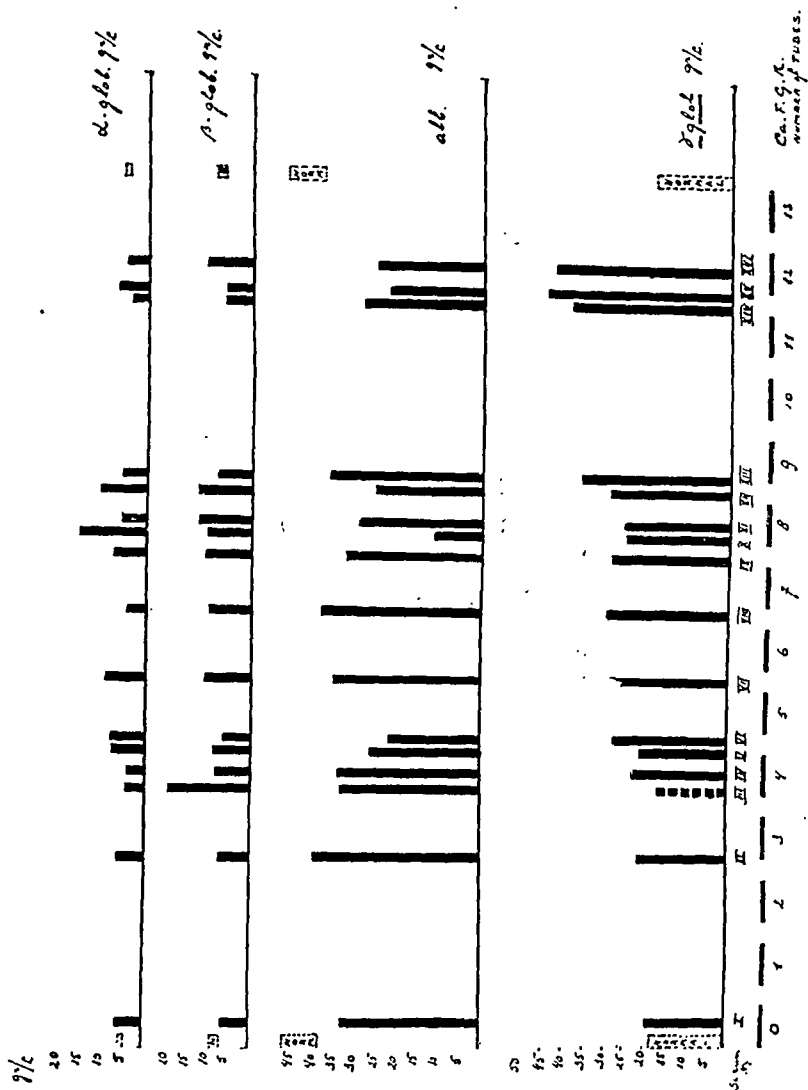
Column 16. Total globulin content (method of Majoor). The normals are calculated from the average figures in columns 11 and 12.

Column 17. Total globulin determined by electrophoresis. The normal figures are derived from the average relative values of Westermann applied to the border values for the total protein in normal cases.

Column 18. Albumin/Gammaglobulin ratio. (Electrophoresis.)

Column 19. The number of tubes showing flocculation in the Mancke-Sommer reaction.

Column 20. One-hour readings of the blood sedimentation rate (Westergren method).



Graph 2. This graph shows the absolute values, determined by electrophoresis, for the various protein fractions of 16 sera, with the corresponding result of the Ca.F.G.R. The absolute values of the various fractions are calculated from the relative values obtained on electrophoresis and the total protein, determined by Kjeldahl's method.

The normal figures are borrowed from Westermann (9), measured on the same apparatus as was used for this investigation and under similar test-conditions.

in Table 1). Apart from the fact that the euglobulin content (Majoor) is here not in agreement with the result of the Ca.F.G.R., it shows an unusually great deviation from the gammaglobulin

content so that there has presumably been some error made in determining the protein spectrum by Majoor's method.

Comparison between the Ca.F.G.R. and the protein spectrum obtained by means of electrophoresis:

By a comparative investigation, Verschure (6) found a correlation between the results of the standard-formol-gel reaction and the gammaglobulin content of sera determined by electrophoresis. Majoor (7, 8) presupposed, and found, on a small number of sera a relationship between the euglobulin fraction, estimated by his method, and the gammaglobulin fraction, while as mentioned above, the Ca.F.G.R. also appeared to show a good correlation with this euglobulin fraction.

On the basis of these two findings it was to be expected that there would be a correlation between the gammaglobulin content of a serum and the strength of the Ca.F.G.R. The sera of 16 patients from the Department for Skin and Venereal Diseases were than investigated electrophoretically.¹ Moreover, the total protein (Kjeldahl) and the protein spectra after Howe and Majoor of these sera were determined. The absolute content of the fractions estimated relatively by electrophoresis were calculated from the total protein measured by the Kjeldahl method.

In table 1 are the results of the comparative investigation, and on Graph 2 these are illustrated graphically.

A good correlation indeed appeared to exist between the gammaglobulin content of a serum and the result of the Ca.F.G.R. Serum 3 is an exception to this, for while electrophoresis showed a normal gammaglobulin content, the Ca.F.G.R. showed gel formation in four tubes (dilution of 85/100). The following explanation of this is, in my opinion, adequate. This patient was suffering from a very extensive xanthoma tuberosum multiplex, with disturbed

¹ Reference should be made to the theses of Westermann (9) and Verschure (6) for a complete description of the apparatus used and the technique followed.

A phosphate buffer solution, pH 7.6, was used. The serum under investigation, taken off while the patient was fasting, was diluted with the buffer solution to a protein concentration of about 20 g/litre. (Determined refractometrically.) This diluted serum was then dialysed against the buffer solution for 24 hours in an ice-box at 4° C. After the serum had been placed in the apparatus a potential of 200 volts, was brought across the electrodes, current of 50 milliamps, and electrophoresis allowed to proceed until the optimum result was obtained, (2 to 3 hours). For optical registration the Philpot-Svensson method, improved by Zernicke and Janssen, was used. The fact that the values found for the gammaglobulin when using this apparatus are higher than those obtained with other apparatus, is explained by the observation that the delta-effect is not separated here from the gamma-top. The results from the negative pole were used in this investigation.

lipoid metabolism, the blood cholesterol measuring 795 mg%. (The normal value is below 250 mg%). As this lipoid is mostly bound to the betaglobulin in the form of so-called lipo-proteins, which migrate at the same speed as the betaglobulin on electrophoresis, one finds in the diagram, that is brought about by refraction, too high a betaglobulin top. This high top is largely caused by these lipoids. By electrophoresis such a serum will show too high a value for the betaglobulin and consequently too low a relative figure will be found for the other fractions when calculated out of the diagram and this serum contains more gammaglobulin than is calculated by electrophoresis. In this way I feel I must explain this Ca.F.G.R. result of four tubes, which conclusion is supported by the composition of this serum obtained by Majoor's method. Here an increase of the euglobulin content was found agreeing well with a Ca.F.G.R. result of gel formation in four tubes. (Graph 1. Serum Z.)

It is not impossible that the other smaller irregularities which appear in graph 2 in the correlation between the gammaglobulin content and the strength of the Ca.F.G.R. are caused by some influence of the other fractions on the result of the reaction. Although several sera give some indication in this direction, the series is too small for deriving a conclusion hereabout.

For clinical purposes we may neglect the possible slight influences of other fractions and come to the conclusion: the Ca.F.G.R. is positive for a serum with a gammaglobulin content raised above the normal, and the strength of the Ca.F.G.R. is a good indication of this gammaglobulin content.

A normal value of 65 to 80 g/litre has been given for the total protein of serum (Kjeldahl method). Westermann (9) found, using the same electrophoresis apparatus as was used in this investigation and under similar conditions, that the normal value for the gammaglobulin is about 17.4 to 23.3 % of the protein. The highest gammaglobulin content that can be regarded as being

normal is thus: $\frac{23.3 \times 80}{100}$, i. e. 18.6 g/litre. As the Ca.F.G.R. is so empirically arranged that a normal serum just gives a negative reaction, we must assume from the above that a gammaglobulin concentration of slightly more than 18.6 g/litre should give a positive result in the first, undiluted, tube.

The serum from patient I (table 1) showed in the Ca.F.G.R., repeated on several occasions, a marked alteration in the viscosity

of the fluid in the first tube, without it fulfilling the criteria for it to be considered as a positive result. In this serum, which thus lay on the borderline, the gammaglobulin content measured 19.6 g/litre. From the above calculation, supported by the findings in serum I, we must come to the conclusion that gel formation occurs in the first tube of the Ca.F.G.R. when the gammaglobulin content of the serum is approximately 19.5 g/litre or higher. From this we are in turn able to calculate the gammaglobulin concentration of a serum, when the Ca.F.G.R. is positive up to the various tubes in the dilution series:

Table 2.

Number of Tubes	Dilution	Gammaglobulin content g/litre
0 (Negative)	—	<19.5
1	Undiluted	19.5
2	95/100	20.5
3	90/100	21.7
4	85/100	22.9
5	80/100	24.1
6	75/100	26.0
7	70/100	27.9
8	65/100	30.0
9	60/100	32.5
10	55/100	35.5
11	50/100	39.0
12	45/100	43.3
13	40/100	48.8
14	35/100	55.7
15	30/100	65.0

By means of this table we should be able to estimate approximately the gammaglobulin content of a particular serum from a given result in the Ca.F.G.R. Table 3 shows the values for the gammaglobulin obtained from the Ca.F.G.R. alongside the values for the gammaglobulin as found by electrophoresis, while the following column shows the difference between the two figures. In the next column are the values for the euglobulin (Majoor's method) and the last two columns show the differences between the euglobulin figures (Majoor) and the electrophoresis results for gammaglobulin, and finally the differences between the euglobulin results and the gammaglobulin as calculated from the Ca.F.G.R.

The figures in column «d» make us come to the conclusion that it is possible, by means of this very simple method, to estimate approximately the gammaglobulin content of a serum.

Table 3.

Serum	Ca.F.G.R.	Gammaglobulin content calculated from Ca.F.G.R.) G/litre	Gammaglobulin (electrophor.) G/litre	Difference of b—c	Euglobulin (Majoor)	Difference c—e	Difference c—b
	a	b	c	d	e	f	g
I	0	< 19.5	19.6	> -0.2	16.0	- 3.6	- 3.5
II	2 $\frac{3}{4}$	21.4	21.4	0.0	25.0	+ 3.6	+ 3.6
III	4	22.9	17.0	+ 5.9	22.8	+ 5.8	- 0.1
IV	4	22.9	23.1	- 0.2	22.3	- 0.8	- 0.6
V	4 $\frac{1}{2}$	23.3	21.1	+ 2.2	26.3	+ 5.2	+ 3.0
VI	4 $\frac{1}{2}$	23.7	27.9	- 4.2	27.0	- 0.9	+ 3.3
VII	5 $\frac{1}{2}$	25.2	28.6	- 3.4	40.0	+ 11.4	+ 14.8
VIII	6 $\frac{1}{2}$	27.0	29.4	- 2.4	31.0	+ 1.6	+ 4.0
IX	7 $\frac{1}{4}$	28.5	28.1	+ 0.4	34.4	+ 6.0	+ 5.9
X	8	30.0	25.2	+ 4.8	36.8	+ 11.6	+ 6.8
XI	8	30.0	25.1	+ 4.9	30.3	+ 5.2	+ 0.3
XII	8 $\frac{1}{2}$	31.2	29.3	+ 1.9	36.0	+ 6.7	+ 4.8
XIII	8 $\frac{1}{2}$	32.0	36.2	- 4.2	39.0	+ 2.8	+ 7.0
XIV	11 $\frac{1}{2}$	41.1	38.7	+ 2.4	30.5	- 8.2	- 10.6
XV	11 $\frac{1}{2}$	41.1	44.9	- 3.8	40.0	- 4.9	- 1.1
XVI	12	43.3	42.3	+ 1.0	47.5	+ 5.2	+ 4.2

The euglobulin content found by using Majoor's method seems to show less agreement with the gammaglobulin values by electrophoresis than the values calculated from the Ca.F.G.R., in connection with which the remarks on page 273 as to the circumstances by which the euglobulin values for this investigation were arrived at, should be borne in mind. The euglobulin estimation by Howe's method (sodium sulphate concentration of 135 g/litre) does not give a result comparable with the gammaglobulin values. One glance at the tables of de Vries (2) and Gutman (10) is sufficient to convince one of this. de Vries gives a normal range of figures of 2.1—5.8 g/litre for this fraction, and Gutman 1.0—4.0 g/litre.

In summing up one must conclude that it is possible, using this Ca.F.G.R., to show the presence of an increase in the gammaglobulin content of a serum in a very simple way, and that, by regular repetition of the reaction during the course of an illness, it is possible to follow the hypergammaglobulinemia and plot the results on a graph.

Hypergammaglobulinemia is a totally non-specific finding which must be presumed to be a manifestation of the activity of a defence-mechanism of the organism under certain pathological conditions.

The expectation may be expressed that with the help of the Ca.G.F.R. and the graphs plotted from its results in many instances useful information can be obtained for the diagnosis, differential diagnosis, prognosis and the assessment of the value of our therapeutic measures, while the graphs may help us to deepen our insight into the course of the disease.

In a number of dermatological and venereal diseases an investigation with the Ca.F.G.R. was carried out on a fairly extensive scale, the diseases including syphilis, lymphopathia venereum, leprosy, lupus erythematosus acutus disseminatus, and pemphigus vulgaris.

For the results obtained and their interpretation reference should be made to the publication concerned (11. 12).

Also, in certain other diseases the Ca.F.G.R. seemed to me to be of value. These other conditions included chronic infections (tuberculosis), liver diseases etc.

Summary.

A new reaction is described, based on the addition of a definite quantity of a mixture of equal parts of specially prepared 12 % calcium chloride solution and 40 % formalin to a serum dilution series, the serum being diluted with normal saline.

This simple reaction, capable of clear and objective reading, is called the Calcium-Formol-Gel Reaction.

By a comparative investigation of 54 sera there was seen to be a close connection between the result of the reaction and the serum englobulin content, determined by Majoor's method.

Similarly, in a series of 16 sera there was found to be a close correlation with the serum gammaglobulin content, determined by electrophoresis, which could be estimated approximately from the result of the calcium-formol-gel test.

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Ventricular Gradient Studies in Positive Hypoxemia Tests.

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Hypoxemia Tests and their Evaluation.

Much work has been done during the last years in order to investigate the latent coronary insufficiency by means of different electrocardiographical methods. Several investigators (1—8) have found the hypoxemia test, in one form or another, most useful for this purpose. Breathing of a gas with low oxygen content throws a certain burden on the cellular respiration in general and that of sensitive organs like heart and brain in particular. Respiration and circulation may to a certain degree compensate for the impaired oxygen supply by increasing the ventilation and the cardiac output, and the tissue metabolism may respond by utilising the available oxygen more completely (increased arterio-venous oxygen difference). When this compensatory mechanism is not sufficient to cope with the induced hypoxemia, either generally or in some localised area of the myocardium, the electromotor forces in the heart will be changed and currents of injury may appear.

Comparison between electrocardiograms taken before and after induced hypoxemia will reveal the changes in the electromotor forces. The »linear» evaluation of the electrocardiograms only

¹ Biörck suggested the study. Jackson and, later, Rohlin have cooperated in the measurement of the gradients. The responsibility for the views expressed in the article is Biörck's.

permits a qualitative judgement of the direction and approximate magnitude of these changes. It was early felt that a quantitative estimation might be of additional use in the evaluation of the test¹ and the establishment of objective criteria.

The papers by Ashman, Bayley et al. (10—18) on the application of modern electrocardiographic theory to electrocardiograms in myocardial disease indicated a way which might provide such a quantitative evaluation of the hypoxemia tests. This procedure was therefore suggested by Biörck (7 b). The present paper represents an attempt to determine the value of vector-analysis in such tests from a limited number of determinations (17 hypoxemia tests judged as positives acc. to Levy's criteria).

Vector Analysis: Definitions.

The theories and the reasoning on the vector expression of electromotor forces of the heart and of the use of the ventricular gradient are chiefly given in the publications by Wilson, Johnston, MacLeod and Barker (9), Ashman, Byer and Bayley (10), Bayley (11—18) and is briefly summarized in the Primer of Electrocardiography by Burch and Winsor (19). The reader is referred to these works. The electrical activity of the heart during stimulation is one of *depolarization* and immediately after that the previous electrical charges are restituted by a process of *repolarization*. The *mean electrical axis* of the particular process may be defined as the mean electromotive force of depolarization (repolarisation), acting in an average direction during the period of activity. Thus it is a *vector* quantity and has magnitude, direction and sense. The mean electrical axis, of course, consists of a representation of the sum of all the *instantaneous* electrical axes

¹ It should, however, not be forgotten that attempts have also been made to standardize the test itself better by variations of the composition of the gas mixture. Malmström (8) has shown that addition of a certain amount CO₂ to the gas mixture gives a more uniform type of ventilation and therefore more equal arterial oxygen tensions than previous methods. Since using oximetric control of the arterial oxygen saturation during our hypoxemia tests we find, that Biörck's (6, 7) previous suggestion of maintaining a steady level of arterial oxygen saturation by variation of the oxygen percentage of the inhaled gas mixture during the test is probably not satisfactory in the 9 % oxygen-method. With inhalation of 9 % oxygen in nitrogen for 10 minutes there is not enough time to keep the patient on a steady saturation level. It is planned to study the different effects on arterial oxygen saturation with our present method of 9 % oxygen for 10 minutes as well as our former method of 10 % oxygen for 20 minutes from this point of view.

during the particular process, which can be derived from the QRS-loop on the screen of a cathod ray oscillograph.

The *ventricular gradient* is a vector expression of the electrical forces appearing during the sequence of depolarization and repolarization of the heart muscle. It would be zero if repolarization exactly retraced the order of depolarization. In practice this is not so and the gradient therefore has magnitude, direction and sense, and is a measure of the lack of uniformity in the effective duration of the excited state in various fronts of the subepicardial and subendocardial layers. Damage to the heart muscle further alters the electrical sequence and changes the ventricular gradient.

The projection of the gradient onto the anterior plane is given by the vector sum of the net areas of the QRST complexes in any two of the extremity leads (preferably lead I and III) using the triaxial reference system based upon Einthoven's triangle. This however is not a true projection of one plane upon another. The limb leads themselves pick up electrical forces in three dimensions since each is derived from the plane of the root of the limb, themselves not uniplaner. As an approximation, however, the limb leads may be considered as uniplaner.¹ In 95 % of normal subjects the gradient lies within a circle of radius 42 micro-volt-seconds (m. v. s.) centred on a point 58 m. v. s. from the origin at an angle of 50 degrees (13). See fig. 2—4.

Ventricular Gradient: Method of Measuring.

In measuring the area of QRST the following rules were observed.

1. The isoelectric line was taken as the line joining the lower border of successive TP segments. The measurement was not attempted in tracings where the isoelectric line was difficult to fix.
2. The areas enclosed by the lower margin of the ECG contour and the isoelectric line were measured, those above the line being regarded as positive and those below as negative.
3. The area under each curve was obtained by the summation of the »rectangles» enclosed by the curve, the isoelectric line and the 0.02 sec. vertical time-markings.

¹ Sulzer and Duchosal (20—23) have shown that this approximation is not too good. However, although not quite satisfactory the described vector analysis is obviously superior to a linear analysis, and until these authors have been able to demonstrate practical means for arriving at a true three-dimensional analysis, it is regarded as justifiable to work with an approximate one.

4. With a standard deflection of $1 \text{ cm} = 1 \text{ millivolt}$ the area was readily expressed in microvolt seconds (m. v. s.), and a correction was introduced where standardisation was not exact.

5. Each reading was the mean of at least three evaluations.

6. The areas under QRS and under T expressed in m. v. s. were projected on the triaxial reference system and the ventricular gradient constructed as their resultant.

Ventricular Gradient: Features in Myocardial Disease.

The ventricular gradient in normals lies, within the limits of Bayley's aforementioned circle, pointing downwards and to the left in the sixth or fifth sextants of the triaxial reference system, *i. e.* from the heart base to the apex. Abnormal T-waves that are purely secondary to changes of the QRS complexes (such as in hypertrophy and transversal position of the heart) will not be reflected by a pathological ventricular gradient, whereas T-wave changes due to delayed repolarization will influence the ventricular gradient in an abnormal way.

Damage to the heart muscle will change the electric activity in the particular area. This change will manifest itself electrocardiographically provided it encloses the epicardial and/or the endocardial surface of the heart. A damaged area hidden in the muscular mass and without access to any surface will not give any sign in the electrocardiogram.

The change in ventricular gradient in case of myocardial damage of the ischaemic type, that approaches either or both of the surfaces, will depend upon the difference in intensity of the ischemia between the epicardial and the endocardial surface. In cases of »pure» local ischaemia (*i. e.* reversible process without structural alterations in the cells) the first change to take place acc. Bayley is a primary T-wave change. The direction of the T-wave change is such as to sweep the ventricular gradient (which is the resultant of a normal QRS vector (\hat{A}_{QRS}) and an abnormal T vector (\hat{A}_T)) into the fifth or the fourth sextant (clockwise) in case of damage in the area supplied by the left coronary artery and into the first or second sextant (counterclockwise) in case of a damage in the area supplied by the right coronary artery. Strictly apical ischemia will diminish the magnitude of the ventricular gradient while retaining a normal direction or — in excessive cases — reverse it into the third

sextant; diffuse basal ischemia on the contrary will increase the magnitude of the ventricular gradient, also with normal direction. This all means that the T vector (\hat{A}_T) is sweeping around on the other side of the ventricular gradient (\hat{A}_{QKST}), provided the QRS vector (\hat{A}_{QRS}) is essentially unchanged.

If, however, the cellular damage becomes more severe,¹ we will encounter the electrocardiographic signs of *injury*. These are preceded, it is thought, by the above mentioned changes ascribed to ischemia, but consist themselves of changes in the S-T (RS-T) segments, due to currents of injury. When the injury chiefly concerns the epicardial surface, the result will be an upward displacement of the S-T-segment. (Bayley claims that prolonged duration of this event as seen in cardiac infarction is consistent with, if not depending upon, the pericarditis connected with myocardial injury reaching the epicardial surface.) At this point in the chain of events the ischemic T-wave changes disappear and the QRS will be altered (deep Q waves). The healing goes the same way backwards, the S-T-displacement disappears, the T-wave change reappears for some time, until finally only QRS changes remain.

The displacement of the S-T-segment due to a current of injury² is maximal, when only one surface (epicardial or endocardial) is involved.

Epicardial and Endocardial Involvement.

The type of electrocardiographic changes usually encountered in myocardial infarction, as a result of disease or produced experimentally, are upward S-T-changes with corresponding T-wave inversion in either lead I and II or lead II and lead III. Although the primary T-wave changes described by Bayley and deduced from the theory are not always observed the explanation in general fits fairly well.

However, electrocardiograms during spontaneous attacks of angina pectoris and in hypoxemia tests frequently behave con-

¹ This does not imply a structural change, only a physiological deterioration.

² There is also another theoretical explanation for displacement of S-T-segments, viz. summation of delayed depolarization and accelerated repolarization. This has not been taken into consideration in the hypoxemia tests, where such an explanation is very improbable. The measurement of \hat{A}_T in our cases therefore is a measurement of $\hat{A}_{S-T} + T$ as an expression of the current of injury.

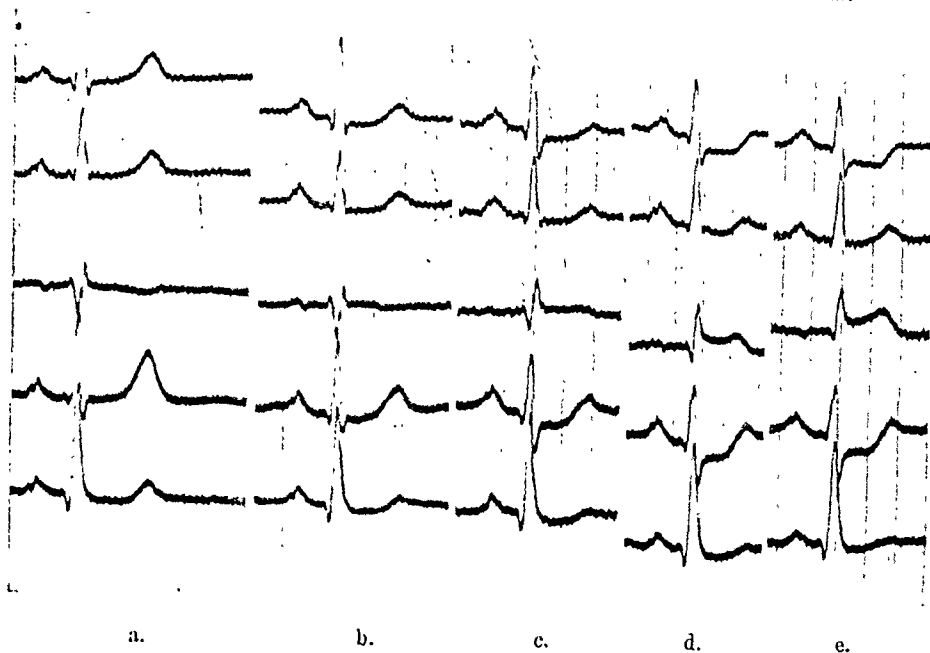


Fig. 1. Case 5.

Tracings represent: a. ECG before hypoxemia.
 b. ECG after 2 minutes of hypoxemia (9% O₂).
 c. ECG after 4 " " " "
 d. ECG after 6 " " " "
 e. ECG after 8 " " " "

The lead are: I, II, III, anterior chest lead, posterior chest lead.

versely, *i. e.* their main feature is a downward displacement of the S-T-segment, with or without changes in the T-waves. As electrocardiograms are usually recorded first some time after the onset of angina and at the end of hypoxemia tests transient T-wave changes may have escaped notice. One of the patients in the present material (case 5), however, was followed with electrocardiograms every other minute during the hypoxemia test (Fig. 1), and no early primary T-wave change was observed.

Some cases even show upward displacement of the S-T-segment, either alone or with downward displacement in some other lead. It is possible that they represent the revival, by means of the induced ischemia area around a silent tissue scar, of an injury current from the borders of the latter. It is our clinical impression that the response with upward displacements may indicate a more »active» condition than the usual downward one.

Theoretically *downward* displacements of the S-T-segment must be considered indicative of *injury to subendocardial layers*. The results of hypoxaemia tests in many cases therefore make it important to investigate the actual behaviour of the ventricular gradient during the test and to study the possible site of the process of ischemia (or injury) acc. to the vector theory advanced by Bayley.

Ventricular Gradient in 17 Hypoxemia Tests.

17 positive hypoxemia tests (evaluated acc. Levy's criteria) from 15 patients were measured with regard to their ventricular gradient before and after induced hypoxemia. They are all briefly recorded in table I.

The ventricular gradient before the test was within normal limits in 11 cases and abnormal in 4 (cases no. 8, 9, 11 and 12). After the test it remained normal in two cases (no. 4 and 15). Another case (no. 8) showed a reversion towards the normal area. The rest of the cases showed a diversion of the gradient into pathological areas.

There was generally a good agreement between the evaluation of the test acc. Levy and the estimation with vector analysis. Only in two cases was there a normal ventricular gradient after hypoxemia (cases no. 4 and 15). In case 4 the ECG was almost a borderline tracing, with an S-T-depression in the limb leads and one chest lead together slightly exceeding 3 mm. It may well be, that the result of the vector analysis is the more correct evaluation of such a test. Case 15 was judged as positive on account of an unusually great S-T-depression in the anterior chest lead of Nylin-Nehb. In case 9 it is obvious from the ECG, that the ventricular gradient before the test was of abnormal magnitude on account of extremely tall R-waves. When the hypoxemia brought about S-T-depressions there was an equalization of the areas QRS and T, which brought the gradient to the area of subnormal magnitude in the sixth sextant. Biörck and Pejme (24) have shown that the existence of unusually tall R-waves in many cases with congenital heart disease requires S-T-depressions and inverted T-waves for the establishment of a normal ventricular gradient.

The direction of the shift of the ventricular gradient was in 5 cases (no. 5, 7, 8, 14 and 15) clockwise, in the other 10 counter-clockwise (figures 2—4). Of the former group cases 5 and 8

Table I.

Case No.	Sex	Age	Diagnosis	Ecg. before	Ecg. after	VG before	VG after	Direction of shift	Remarks
1	F	28	Congen. heart disease	N	AB. ST ₁ ↓ ST ₂ ↓ ST ₃ ↓ ST _{ant} ↓	N 60 mvs + 40°	AB 8 mvs — 30°	↖	Abbreviations: Ecg = electrocardiogram
2	F	52	Cardiosclerosis	LAD, N	AB. ST ₁ ↓ ST ₂ ↓ ST ₃ o ST _{ant} ↓	N 65 mvs + 15°	AB 14 mvs 0°	↖	VG = ventricular gradient
3	M	61	Angina pect.	N	AB. ST ₁ ↓ ST ₂ ↓ ST ₃ ↓ ST _{ant} ↓	N 67 mvs + 70°	AB 33 mvs — 105°	↖?	F = female
4	M	40	Aortic stenosis	N?	AB. ST ₁ o ST ₂ ↓ ST ₃ ↓ ST _{ant} ↓	N 57 mvs + 45°	N 25 mvs + 30°	↖	M = male
5	M	54	Angina pect. ^{13/12} 46	LAD, N	AB. ST ₁ ↓ ST ₂ o ST ₃ ↑ ST _{ant} ↓	N 38 mvs + 20°	AB 52 mvs + 115°	↙	N = normal
»	»	»	Angina pect. ^{4/3} 47	LAD, N	AB — » —	N 43 mvs + 20°	AB 54 mvs + 105°	↙	AB = abnormal
»	»	»	Angina pect. ^{10/1} 47	LAD, N	AB — » —	N 30 mvs + 30°	AB 65 mvs + 95°	↙	Ant = anterior chest lead
6	F	45	Hypertension	LAD, N	AB. ST ₁ ↓ ST ₂ ↓ ST ₃ ↑ ST _{ant} ↓	N 72 mvs + 21°	AB 25 mvs + 8°	↖	Post = posterior chest lead
7	F	50	Hypertension + Angina pect.	N	AB. ST ₁ ↓ ST ₂ ↓ ST ₃ o ST _{ant} ↓	N 62 mvs + 38°	AB 9 mvs + 128°	↙	mvs = microvolt-second
8	F	44	Coarctation of the aorta	LAD, P ₃ and T ₂ neg.	AB. ST ₁ ↓ ST ₂ ↓ ST ₃ ↑ ST _{ant} ↓	AB 84 mvs — 27°	AB 80 mvs — 14°	↙	LAD = left axis deviation
9	F	43	Patent ductus Botalli	N? Tall R ₂	AB. ST ₁ ↓ ST ₂ ↓ ST ₃ ↓ ST _{ant} ↓	AB 112 mvs + 62°	AB 20 mvs + 10°	↖	RAD = right axis deviation
10	M	67	Cardiosclerosis	LAD, N	AB. ST ₁ ↓ ST ₂ ↓ ST ₃ ↑ ST _{ant} ↓	N 75 mvs + 78°	AB 20 mvs — 130°	↖?	↑ = upwards, ↓ = downwards, the number of arrows denoting severity
11	M	73	Cardiosclerosis	LAD, N? (ST ₂ ↓)	AB. ST ₁ ↓ ST ₂ ↓ ST ₃ o ST _{ant} ↓	AB 18 mvs + 23°	AB 9 mvs — 145°	↖?	= counter-clockwise
12	F	63	Mental depression	LAD, N	AB. ST ₁ ↓ ST ₂ ↓ ST ₃ o ST _{ant} ↓	AB 104 mvs + 40°	AB 7 mvs + 16°	↖	= clockwise
13	M	39	Mitral stenosis	RAD, T ₂ di-phus. T _{ant} ↑	AB. ST ₁ o ST ₂ ↓ ST ₃ ↓ ST _{ant} ↑	N 44 mvs + 68°	AB 14 mvs — 85°	↖	
14	F	55	Hypertension + Angina pect.	LAD, N	AB. ST ₁ ↓ ST ₂ ↓ ST ₃ o ST _{ant} ↓	N 35 mvs + 35°	AB 3 mvs + 90°	↙	
15	F	50	Cardiosclerosis + Hypertension	LAD, N	AB. ST ₁ ↓ ST ₂ ↓ ST ₃ o ST _{ant} ↓	N 70 mvs + 45°	N 48 mvs + 53°	↙	

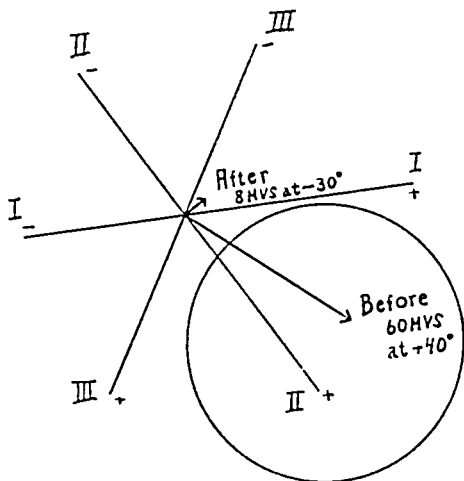
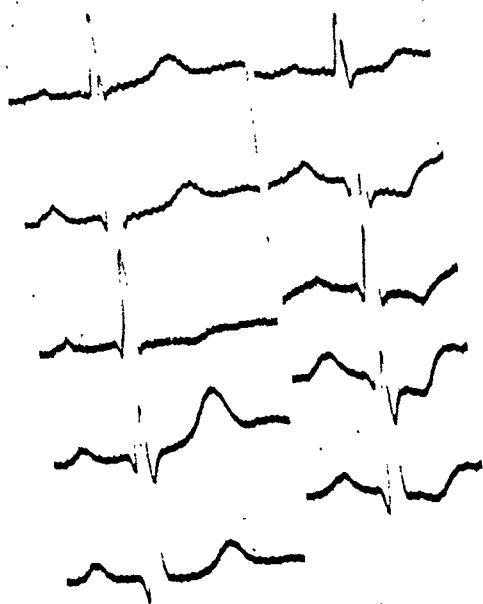


Fig. 2. Case 1.

are the only two cases in which discordant displacements of $S-T_I$ and $S-T_{III}$ were observed, $S-T_I$ being depressed and $S-T_{III}$ being elevated. Case 7 did not show these features, only a very marked depression of $S-T_I$ and $S-T_{II}$. In the cases 14 and 15 there was a moderate depression of $S-T_I$ and $S-T_{II}$. Where T_I becomes negative the question of the shift clockwise/counter-clockwise is chiefly depending upon the \hat{A}_{QRS} in relation to the \hat{A}_T , when the latter has moved to the third sextant. Very small differences in the angle of \hat{A}_{QRS} or \hat{A}_T will usually, theoretically, suffice to change the manifest shift of \hat{G} from clockwise to counter-clockwise and vice versa. It is admitted that deviation of the gradient into the third sextant is difficult to denote as clockwise or counter-clockwise, unless serial tracings during the test are made and measured. Where the projection on the frontal plane of the three-dimensional (spatial) vectors is largely incorrect (as in very transverse or rotated hearts) the values may be less reliable.

One of the remarkable findings is thus that in all cases, electrocardiographically denoted as showing »posterior coronary insuffi-

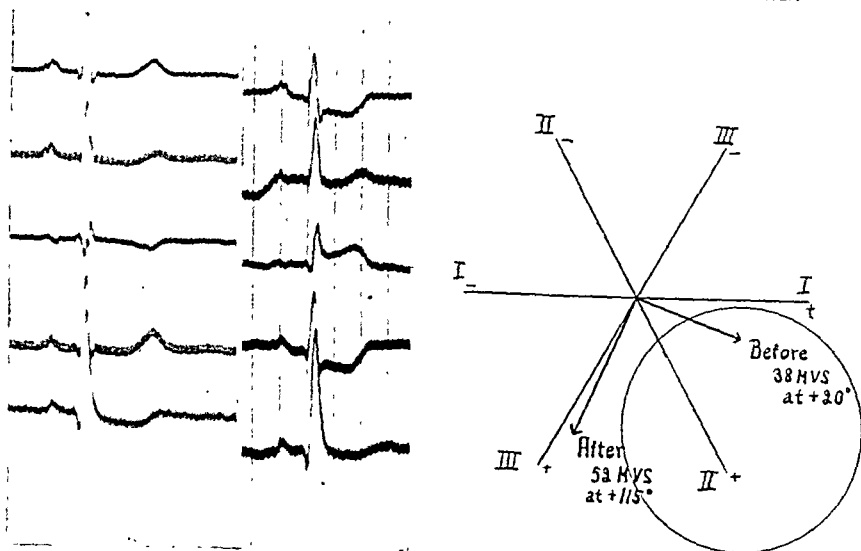


Fig. 3. Case 5 a.

ciency» the shift has been counter-clockwise, whereas in the cases denoted as »anterior coronary insufficiency» the shift has been clockwise in three and counter-clockwise in five. In case of upward displacement of the S-T-segments (»subepicardial damages») clockwise rotation of the ventricular gradient is said to mean impairment of the circulation through the left coronary artery (»anterior coronary insufficiency»), while a counter-clockwise shift will mean impairment of the circulation through the right coronary artery (»posterior coronary insufficiency»). There is, apparently, with regard to the S-T-depression pattern a contradiction between the evaluation of the electrocardiographical tracings and the vector analysis thereof. It is possible that the concordant—discordant behaviour of the S-T-segments points to a difference, much more fundamental than the depression of this or that S-T-segment.

Several tests, judged as positives acc. Levy were not measured, either for technical reasons or because the main ECG-changes were observed in the chest leads rather than in the limb leads. It is therefore clear, that the estimation of ventricular gradient cannot for routine purposes replace the usual linear evaluation of the test. This evaluation has, however, no theoretical basis whatsoever; it is merely a convention, that has shown unexpectedly good agreement (6, 7) with the prognosis as judged from a comparison with postmortem examinations. Now, it is apparent

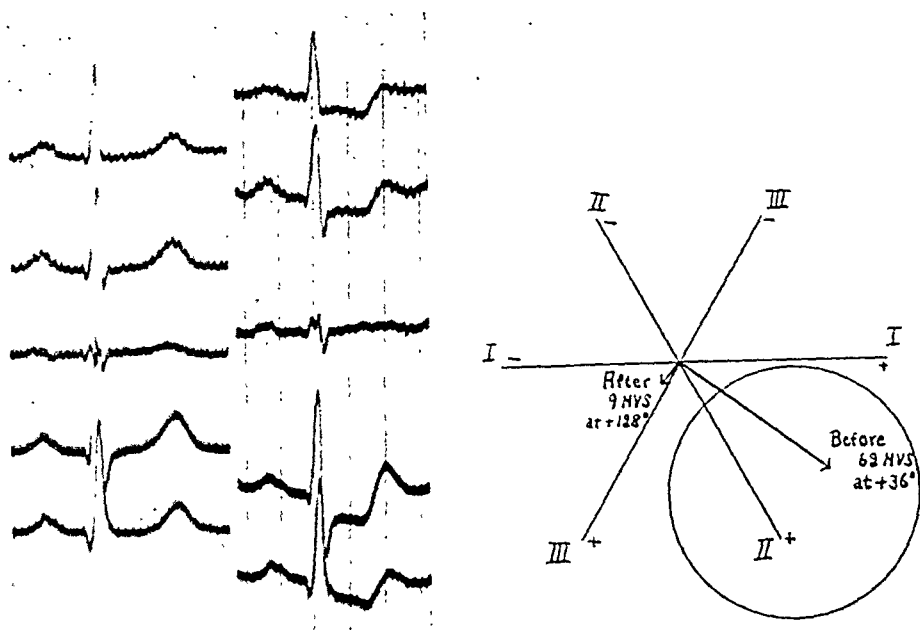


Fig. 4. Case 7.

from the previous discussion and the results here mentioned that we do not know, what the different S-T-depressions really signify. Two things are obvious: 1) cardiac infarction brings about upward S-T-displacements, indicating chiefly subepicardial injury, 2) angina pectoris and hypoxemia tests bring about downward S-T-displacements, probably indicating chiefly subendocardial injury.

Earlier authors have pointed to the hypoxemia test as an almost exclusive method for the diagnosis of coronary insufficiency of the organic (arteriosclerotic) type. One has thought that the changes brought about by induced hypoxemia disclosed the latent coronary insufficiency, which would later become manifest.

Increased experience with different functional conditions and also with hypoxemia in children (Mannheimer, 25) has made this idea somewhat vulnerable. The vector analysis, as shown above, also undermines that conception, as it is apparent that the changes in the electrocardiogram during hypoxemia are generally not of the same kind as those encountered in an eventual subsequent infarction. (It is, however, quite possible that there is in some cases a close uniformity between the ECG changes during hypoxemia and those during attacks of spontaneous

angina pectoris.) The question therefore must be discussed: What is the significance of the S-T-depression of the type generally encountered during positive hypoxemia tests?

Hypoxemia Tests and Injury-against the Rule.

Our studies with the oximeter (26) have established, that the decrease in arterial oxygen saturation during the hypoxemia test is a gradual one and showing great individual variations. It is therefore reasonable to suppose that the heart muscle in different patients at the end of the test, as well as the heart muscle of the same patient during the test, may arrive at various stages of ischemia, some of which will pass further on into the stage of injury. It would therefore also be reasonable to expect more electrocardiographic signs of ischemia than such of injury (acc. to the definitions given by Bayley). The opposite is however the case. Isolated primary T-wave inversions are hardly ever seen; T-wave inversions are practically always accompanied by S-T-depressions and are probably part of the same picture. Nor is there any evidence of increased magnitude of previously positive T-waves.

As far as we can find, therefore, the heart muscle in hypoxemia tests usually passes gradually into a state of »injury» without passing through any previous »ischemia». It is doubtful, then, whether this particular »injury» pattern really means an injury in strict sense or if it means something different.

These difficulties led one of us (G. B.) already early in 1946 to enter a discussion with Dr. Bayley regarding the significance of the downward S-T-segment pattern. The following answer by Dr. Bayley (27) may be quoted, as it illuminates the difficulties and different possibilities in the explanation:

— — —

With regard to the majority of attacks of angina pectoris occurring in the third, fourth, and possibly fifth decade, recurring over a period usually of years, the majority of which give a positive anoxemia test or show similar electrocardiographic changes during an actual spontaneous attack, the R-ST junction displacements are downward in L I and L II, most marked in L II, and are variable, either upward or downward, in L III. These displacements are reversible with nitroglycerin. In multiple precordial leads recorded at the same time when the limb leads show the downward displacement, a negative R-ST junction displacement is likewise observed from that region of the precordium at which the left ventricle trans-

mits its potential variations. These displacements may be very striking. They are usually quite transient, lasting for several minutes, but in rare instances may be prolonged, and the attack fatal.

Here, when the axis of injury, E_I , is plotted on the triaxial reference system, it is directed into the third sextant along the negative path of the L II line. The T-waves, when apparent, tend to peak in the opposite direction to that of the R-ST displacement.

In the article »On Certain Applications of Modern Electrocardiographic Theory . . .» (13), the rule given for the ordinary R-ST junction displacement as determined by the triaxial reference system is that E_I has the direction of a line drawn from the center of the involved ventricle toward the center or centroid of the injured region. In the case of the above cited injury effects, this line would be approximately parallel to the negative half of the L II line, or in the adjacent halves of the second and third sextant. In three dimensional space, the direction of this line is from the center of the left ventricle toward the valve opening where no muscle exists. It is, therefore, obvious that this rule does not hold. If it is postulated that the endocardial surface is more intensely injured than the epicardial surface, a rule may be formulated for the axis of injury which gives it the direction of a line drawn from the center or centroid of the injured region toward the center of the involved ventricle (injury-against-the-rule).

The appearance of a more or less diffuse subendocardial injury will have the geometrically opposite effects, as compared to that of pericarditis which injures the subepicardial muscle. The centroid of the former distribution of injury is near the apex of the ventricular chamber, and a line drawn from this point to the center of the ventricle will have an apex—base direction and when projected onto the triaxial reference system will fall into the adjacent halves of the second and third sextant, and when projected from here onto the three reference axes will indicate a large downward displacement of the R-ST junction in L II and a somewhat smaller downward displacement in L I, with a variable displacement in L III, small, and either positive or negative. A diffuse subendocardial ischemia of this kind could, I believe, be produced under the considered clinical circumstances only by a generalized coronary spasm in a coronary arterial tree, the walls of which are, generally speaking, limber. The depth of the subendocardial ischemic region in the direction away from the endocardium should be greater in the apical region, more distant from the source of blood supply. During the attack of spasm, there are apparently two times intervals at which ventricular fibrillation is likely to occur, *i. e.*, within five or ten minutes after the spasm begins and again immediately upon re-irrigation with blood of the ischemic region. If the patient dies at this time, the heart may show little or nothing. In some cases where death is not immediate and the spasm is prolonged, the distribution of the ischemic region will be defined by the early changes of necrosis of the muscle. I have had opportunity recently of following such a case. The electro-

cardiogram and the distribution of the necrotic muscle conformed to these ideas. The coronary arteries were normal.

Dr. Bayley's letter continues by a short comment on two cases in an article by Thomson and Feil (28), which are also briefly discussed in one of his papers (18) and summarizes his opinion as follows:

Injury-against-the-rule occurs, in so far as I have been able to determine, in these two groups of patients, one in which there is a generalized subendocardial ischemia due to generalized spasm of the coronary arterial tree, and the other in which a rather extensive atherosclerotic coronary arterial tree is present and in which there is a local ischemia of the ventricular wall with a more intense involvement of the endocardial surface compared with the neighbouring epicardial surface as a result of impaired collateral irrigation.

In the abovementioned paper (18) Bayley discusses the two cases from the work by Thomson and Feil (28). From calculations of the S-T-displacements in these two cases of infarction of the lateral wall of the left ventricle Bayley concludes that the infarctions in question were predominantly at the endocardial side of the heart muscle. At least in one of the cases this assumption was verified by Feil. In this connection Bayley makes the following comments:

The subendocardial arterial plexus of Gross is apparently a much more important factor in sustaining the endocardial lamina of a region robbed of its natural blood supply than is imbibition or reversal of flow through thebesian veins. If this opinion is accepted, it may be observed further that survival of the subendocardial lamina (under an infarct) which accounts for injury-with-the-rule, depends largely upon an adequate collateral circulation at the time of the attack of infarction. Moreover, if an adequate collateral circulation is not available, the subendocardial lamina must, it seems, undergo necrosis along with the superjacent muscle, and the net result is a greater damage of the local subendocardial, than of the local subepicardial, laminae. In the event of a generalized coronary artery spasm, the plexus of Gross cannot operate effectively, and the electrocardiographic pattern of injury-against-the-rule occurs as depicted in your article (by dr. Feil) of 1928.

Insofar it regards the importance of the different ways of nutrition of the subendocardial layers this idea is consistent with the findings reported by Mallory, White and Salcedo-Salgar (29) from a post-mortem study of cardiac infarction, which showed, that the uniform necrosis of the heart muscle after infarction

failed to involve a thin layer of surviving muscle (0.3 to 0.5 mm thick) which lay beneath the endocardium and extended within the wall along the thebesian veins of the involved region.

It is of importance to add, that Wolferth, Bellet, Livezey and Murphy (30) also report, that extensive trauma to the endocardial side of the myocardium produces a negative RS-T segment potential change at the ventricular epicardial surfaces. Dearing, Barnes and Essex (31) have also found, that digitalis effects (which manifest themselves in the ECG by S-T-depressions) are exerted preponderantly on the endocardial side of the cardiac wall. From all this there seems to be little doubt left, that the effect of induced hypoxemia is an ischemia (or »injury») chiefly of the endocardial side of the heart muscle. Why then is the endocardial side of the heart muscle more susceptible to hypoxemia than the epicardial part of it? One possible explanation is the following:

In coronary artery occlusion the epicardial part of the muscle distal to the occlusion is entirely dependent on collaterals for continuation of its blood supply. The sub-endocardial layers, however, may still receive a certain blood supply from the different channels which communicate with the ventricles themselves.¹ For this reason the damage to the subepicardial layers in case of insufficient collaterals will almost always be greater than that suffered by the subendocardial layers, and, consequently, an injury-with-the-rule will appear in the electrocardiogram.

When the arterial blood is insufficiently saturated with oxygen, as in hypoxemia tests, the condition are changed. The oxygen saturation of the blood in the left ventricle is markedly decreased, and in this case the oxygen saturation of the blood in the sub-endocardial network may not be sufficient to counteract a process of ischemia, due to impaired circulation through some coronary artery together with the deficient oxygen saturation of the blood passing through this artery. Because of their priv-

¹ These channels have been recently carefully studied by, among others, Wearn and D. E. Gregg (32, 33), Printzmetal and Simkin (34) and in our country Engstrand (35). These investigations all confirm the existence of different communications between the coronary arteries and veins and the heart chambers (arterioluminal vessels, thebesian veins and arteriosinusoidal vessels). The number of communications is relatively greater in the right heart. The blood flow in these vessels is usually directed from the coronary artery to the heart chamber. In pathological conditions, however, it is experimentally proved that a retrograde flow is possible, and cases are known, where the heart has sustained an active life with the mouths of both coronary arteries occluded.

ileges during normal conditions the subendocardial layers may even be especially sensitive to this abrupt and abnormal form of stress. Whether the hypoxemia also (or chiefly) works by eliciting a generalized coronary artery spasm, as suggested by Bayley in angina pectoris,¹ is naturally difficult to determine. The results in some cases of sympathicotonia could however be fairly well understood by such an explanation (36).

A conception of the mode of action of the hypoxemia test along these lines may explain, 1:o why positive tests of the subendocardial variety can be encountered in cases where no coronary arteriosclerosis is suspected, 2:o why the tests are of a subendocardial variety even in cases where an arteriosclerotic coronary disease is highly suspected or even certain (viz. previous myocardial infarction). It might also give a clue to the understanding of the usually negative hypoxemia tests in cases with congenital heart disease of the cyanotic variety such as the tetralogy of Fallot's or the Eisenmenger complex, which have been observed by us (40) on a more or less adult material and by Mannheim (39) on children. It may be that the subendocardial layers of these patients, who are accustomed to a deficient oxygen saturation of the blood in the left ventricle, are less sensitive to a sudden decrease in oxygen saturation and therefore less liable to show subendocardial injury.²

Some few words should be said regarding the direction of the shift of the ventricular gradient. It was previously mentioned, that there was no definite correlation between the direction of the shift and the type of S-T-depression. Although Bayley's statement, that the depression should be greatest in lead II and smaller in lead I, and that lead III would show any kind of small displacement proves correct in many cases, it is by no

¹ This explanation for the ECG-changes in spontaneous angina pectoris may prove correct, but the immediate evidence for it is not too great. It still seems more logical to assume the following pathophysiological chain of events, viz.: relatively insufficient blood flow through an arteriosclerotic coronary artery → ischemia distally, esp. subepicardially, if collaterals are insufficient (= negative T-wave!) → pain (see Lindgren's study, 37) → generalized coronary artery spasm or even general arterial spasm (elevated blood pressure!) → spasm of subendocardial arterial plexus → subendocardial ischemia (injury-against-the-rule, S-T-depression). This would be consistent with Bayley's suggestion, provided a primary T-wave change is observed. Increased intraventricular and intramyocardial pressure may eventually also be a factor to consider in angina with elevated blood pressure, in accordance with views, expressed by Lepeschkin (38).

² It might be of interest to study the thebesian vessels etc. of the heart in cyanotic congenital malformations.

means without exceptions. Further on in his letter he states, that the electrocardiographical effects of subendocardial injury will have the geometrically opposite effects as compared to a subepicardial injury. It would then be interesting to investigate the correlation between the electrocardiographic findings in positive hypoxemia tests and the findings at necropsy. Case 15 seems rather suitable for the purpose, as this patient (female hypertensive) died of myocardial infarction 3 months after a hypoxemia test.

The electrocardiogram at rest showed left axis deviation, but otherwise nothing abnormal. The ventricular gradient was entirely within the normal area. After 6 minutes of hypoxemia, when the patient experienced severe pain, because of which the test was interrupted, there was a depression of $S-T_I$, $S-T_{II}$ and $S-T$ in the anterior chest lead. T_I and T_{Ant} were diphasic, probably as a result of the marked $S-T$ -depression. The ventricular gradient after the test showed a moderate clockwise shift, but was still entirely normal as to direction and magnitude. Evaluated acc. Levy the test was strongly positive on account of very marked $S-T$ -depression in the anterior chest lead.

After a period with symptoms of progressive coronary sclerosis the patient succumbed to a myocardial infarction three months later. About one month before death the ECG, which was in the meantime essentially unaltered, showed markedly inverted T_I (ischemia) and about one week later there was a picture of anterior infarction with elevation of $S-T_I$ and depression in all other leads. This ECG-type persisted, with signs of healing, for three weeks until the patient died in progressive cardiac failure.

The autopsy revealed: Marked general arteriosclerosis. The right coronary artery showed moderate arteriosclerosis but had a good lumen throughout. Both branches of the left coronary artery were very arteriosclerotic and the circumflex branch was filled with a recent thrombus. The septum, the anterior and the lateral wall of the left ventricle showed a very extensive recent myocardial necrosis. There is unfortunately no comment regarding its distribution (epicardial and/or endocardial). The clockwise rotation of the ventricular gradient during hypoxemia in this case, anyhow, seems consistent with the finding of an anterior (-septal-lateral) myocardial damage.

Final Comments.

This paper has been intended as a participation in two discussions, viz. on the vector analysis of the «coronary» electrocardiogram and on the significance and usefulness of hypoxemia tests. It does not intend to present any final statements,

but represents an attempt to arrive at some clarity in these fundamental questions. It has been worked out from the analysis of a material at hand. It is hoped, that it will arouse some interest in an experimental approach to the problems, whereby it would be possible to vary the conditions more or less at will and thereby perhaps to throw more light on processes, which we now record only partly and perhaps under far from ideal conditions.

Summary.

Experience with the hypoxemia test has stimulated our interest in finding a theoretically sound mathematical method for the quantitative evaluation of the electrocardiograms before and after breathing a low-oxygen gas mixture. The possibilities of vector analysis (determination of especially the ventricular gradient) have been investigated in 17 positive hypoxemia tests from 15 patients. The induced hypoxemia in most cases caused a shift of the ventricular gradient into a pathological area. The significance of the shift is discussed, and it is assumed that the downward displacement of S-T-segments seen in such tests is due chiefly to subendocardial injury. The rationality of this conception is discussed and it is felt that it may explain some hitherto disturbing observations. The fundamentals of the vector theory when applied to this particular field however do not altogether fit with the actual observations. Further studies, especially experimental ones, are therefore necessary.

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Book Review.

Tillier, Henry: Anatomie radiologique normale. 234 pp. 350 figs. Price: 600 frs. G. Doin & Cie, Paris 1947.

This book is an elementary introduction to normal roentgen anatomy. The greater part of it is devoted to the skeleton, and in this section the author makes detailed analyses of the contours in ordinary projections. The value of the book lies in this section, and it would have been an advantage if it had been limited to this field. The other parts are devoted to the internal organs; they are treated very cursorily and are of no immediate interest, except to those who wish to read a very concise summary of such things. On the whole the statements in the book are reliable, though objections may be raised against one or two, for instance the statement that the gall-bladder is not visible under X-rays unless it is filled with contrast. A couple of pages are devoted to encephalography, but they give no indication of any acquaintance with this method of examination.

The book has one great defect: there are no reproductions of X-ray pictures. The illustration material — which is abundant — consists of drawings. In the part dealing with the skeleton they are on the whole often instructive; in the other sections of the book they are often too small and crowded. The author says in the preface that a study of roentgenology resolves itself into two parts: 1) to see, 2) to try to understand what is seen. This is correct enough, but a roentgenological guide which is illustrated only with drawings and lacks reproductions of roentgen pictures does not convey this desirable knowledge. It only gives information as to what the author considers himself having seen in his pictures.

Ingemar Hessén.

From Lyster Sanatorium, Norway.
(Director: Chief Medical Officer T. Gjessing.)

Investigations on the Respiration in Patients with Lung Tuberculosis by Short Transitory Work.

By

WILHELM BJERKNES †.

District Medical Officer.

(Submitted for publication March 6, 1948.)

Preface.

The investigations here described were commenced in the summer of 1939 by Wilhelm Bjerknes, District Medical Officer, with the two undersigned as assistants. The author at that time was acting as Assistant Medical Officer at Lyster Sanatorium.

Through the kindness of, and the great interest shown by Dr. Gjessing, the Chief Medical Officer, the best possible working conditions were provided, the investigations being assisted by grants from The Scientific Research Fund of 1919 (Det Videnskabelige Forskningsfond av 1919) and Malthe's Surgical Endowment (Malthes Kirurgiske Legat).

When the author died after a short illness in the summer of 1940, the collection of material was far advanced and it was thereupon decided that we, his assistants who had taken part in the investigations from the commencement, should continue the work until the collection of the material was complete. The work of collecting material was accordingly continued at Lyster.

We attempt below to make public the comparatively large material now available in respect of the respiratory values of patients and normal persons. In the first part of the work we explain the methods employed and we reproduce the numerical

results and experiences gained during the experiments. In the sections »Own Investigations» and »Discussion», we have attempted to collocate the results in accordance with the lines the author has laid down, well knowing, however, that we are not in a position to utilise the material with the clear perception the author himself would have used.

It has not been possible to us to surmount a study of the large mass of literature available regarding working experiments in this field; we therefore mention only a few research workers whose results have a close affiliation with the author's own investigations.

To those who, besides Dr. Gjessing, have assisted us in this work, we would express our thanks, in the first place to Dr. Konrad Birkhaug who helped us in planning the statistical treatment of our material, and further also to O. Espensen, Chemist, and Jens Vilhelm Poulsen, student of science, for their valuable help. The editorial side of the work has been considerably lightened by the great interest shown by Professor Dr. Kristine Bonnevie in perusing and arranging the entire manuscript.

Oslo 1945.

Odd E. Hanssen,
Medical Student.

Ragnwald K. Thorsen,
Medical Student.

Introduction.

In clinical examination of pulmonary diseases particularly in tuberculosis of the lungs, a simple and convertible function-test is needed, and although numerous investigations have in the course of years been carried out in this field, no particular standard method has gained ground.

During recent years, however, spirographical investigations in air and in oxygen have been much used as function-tests, especially in pulmonary tuberculosis. The author, who worked in accordance with this method partly with improved methodics, came, however, to the following result (Bjerknes 1939 p. 471):

»Einzelne Beobachtungen sowohl über die Sauerstoffaufnahme als auch über die Ventilation werden praktisch illusorisch gemacht durch die starke individuelle Variation. Bei der Ermittlung der Sauerstoffaufnahme wirkt sich zudem noch die Grösse des methodischen Fehlers ebenso aus. Wenn man auch die zu diesen

Untersuchungen benutzte verbesserte Methodik, mit langen Luftkurven und Sauerstoffprozenten, die der atmosphärischen Luft sehr nahe liegen, anwendet, kann der Funktionsprüfung für den einzelnen Patienten kaum praktischer Wert beigelegt werden.»

In order if possible to get closer to the question of a simple lung function-test, it was planned to investigate on a broad basis the function of the tubercular lung, under circumstances as physiological as possible. This was rendered possible by an apparatus constructed by Dr. Scholander with whom the author was in intimate co-operation.

This apparatus permits of protracted continual registration of the respiration in atmospheric air, where the carbon dioxide output, the acceptance of oxygen and the ventilation are measured spirographically. By this method, the respiration was continually registered during rest as well as during and after a brief standardised and measurable task. A working-time of one minute was chosen — so short that the organism had insufficient time to adapt itself to the increased demand. Scholander had discovered that the respiration curves after such work assumed a characteristically changed course, certainly partly dependent upon the anaerobe work which takes place during the commencement of muscular work. As it has also been found that the lactic acid concentration of the blood was dependent upon the same anaerobe work, it was the author's intention to investigate as to whether conclusions could be drawn from such curves regarding the lactic acid concentration in the blood, without having to recourse to blood-tests. He assumed that in tuberculosis, owing to the anatomical changes in the lungs, variations are also to be found in the anaerobe work with consequent change beyond the normal in the lactic acid concentration of the blood. The corresponding alterations in the gaseous exchange in the lungs would in this case be determinable by Scholander's apparatus. On the basis of such broadly planned investigations, the author hoped possibly to be able to find a respiratory condition easily to be registered and characteristic enough to be used as a function-test.

Technique.

In the investigations discussed below, Scholander's »gas-layering» apparatus (Fig. 1) has been used. This apparatus has been described by Scholander (1937); it works with atmospheric air,

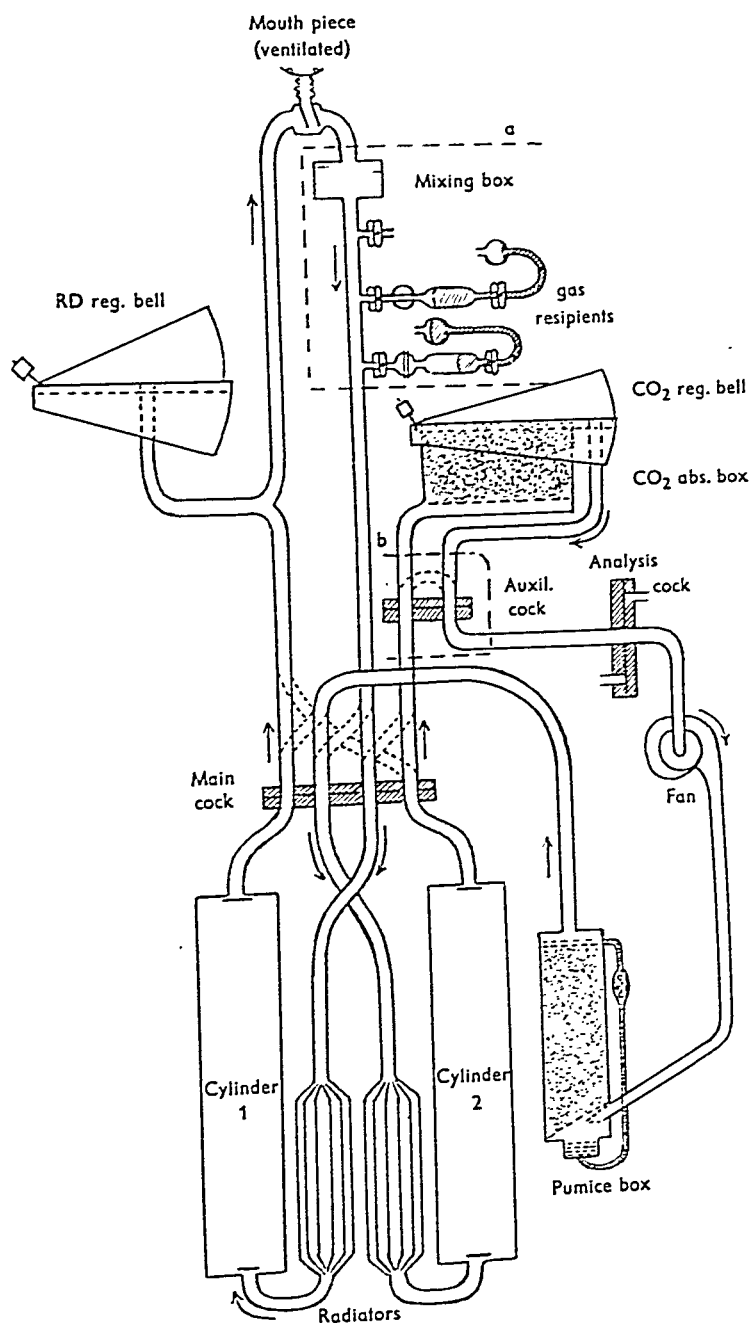


Fig. 1. Scholander's *Gas-layering apparatus* (from Scholander 1937, fig. 2, p. 54) as modified by Bjerknes through addition of the parts included in a and b.

allows of tests of long duration and registers the following data direct:

- 1) A *respiratory difference-curve* (RD) which expresses at any given time the difference between oxygen acceptance and carbon dioxide excretion.
- 2) *Carbon dioxide output* in 4 minutes periods.
- 3) *Ventilation* registered direct by a special ventilation indicator, constructed by the author and described below (Fig. 2).
- 4) A *Base-line* where the length of the periods is indicated.

A brief explanation of the principle of these investigations is given below.

Scholander's Apparatus (Fig. 1): Inspiration is from the top and expiration is through the bottom of a cylinder (*Cylinder 1*), which takes about 60 litres and is filled with atmospheric air. A valve ensures a uniformly directed air current. A *registration bell* (RD bell) is coupled to the inspiration-tube by means of a simple lateral connection. The CO_2 contents of the expiration air make this heavier than the atmospheric air and it is stored in the cylinder from the bottom upwards. The RD-bell registers every expiration and inspiration and draws the *respiratory-difference curve*. Before the expiration-air reaches the top of the cylinder, a new cylinder (*Cylinder 2*) filled with atmospheric air is coupled into the circle by a cock (*Main cock*), and expiration continues uninterrupted. Simultaneously the first cylinder is coupled by the same cock to an absorption circuit where a *fan* circulates the air and where, after the opening of an *analysis cock*, the CO_2 is absorbed by soda lime (*CO_2 abs. box*). The amount of CO_2 absorbed is registered by a clock (*CO_2 -bell*) inserted in the circuit. A *pumice box* moistened with water ensures constant vapour tension. When absorption is complete the cylinder is aired by and filled with atmospheric air. The whole apparatus is thereupon placed in a water-bath and the expiration-air assumes the temperature of the water-bath on passing through a radiator.

As mentioned, the author constructed a ventilation indicator which was used instead of that found originally on the apparatus. It is simple and accurate and draws a continuous curve from which the ventilation may be read at any time.

The *indicator* (Fig. 2) consists of a small light wheel on the edge of which is a track and alongside of this a row of small, tightly placed teeth. A hook catches into the teeth whereby the wheel can only revolve in one direction. A *registrering-pen* is fixed

excentrically to the wheel. Fastened to the RD-bell is a thin cord lying in the track and kept taut by a small weight. With the sinking of the RD-bell at every inspiration, the wheel revolves corresponding to the fall of the bell; with the rising of the bell

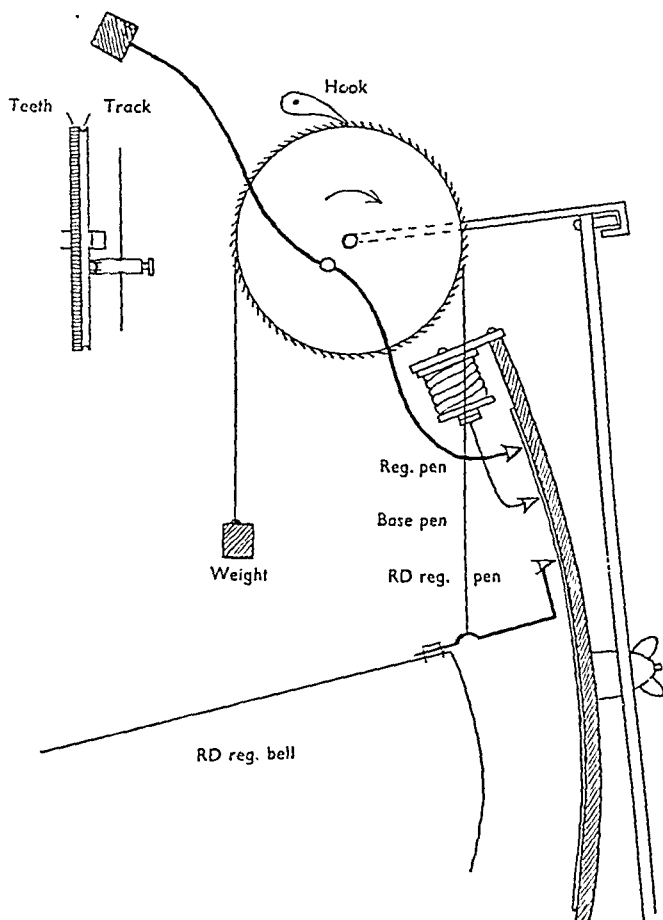


Fig. 2. *Ventilation indicator*, constructed by W. Bjerknæs. Explanation in the text.

upon expiration, the cord is drawn back by the weight, the wheel meanwhile standing still. In this manner a wave-curve is noted (see fig. 3) which represents the sum total of all inspirations. The ventilation indicator is calibrated to accord with the RD-bell, one revolution of the wheel representing a ventilation of 10 l. The registering-pen is placed vertically above the RD-bell-pen and the pen drawing the base line, and the ventilation can thus be read off for any section whatsoever of the respiration curve.

Test Arrangement. The respiration of the test person is registered at rest, during a brief working period and during restitution after work. No one was prior-trained, but merely instructed as to the procedure of the test. No regard was paid to the taking of nourishment, but several tests with the same person were, so to speak, always carried out at the same time of the day. The test-person was placed on a cycle saddle with a chair-like arrangement enabling him to sit comfortably and relaxed. After half an hour in such a position, the test-persons were connected with the apparatus by means of a mouth-piece, the nostrils being closed with a clamp. Then followed 16 minutes registration in a resting position after which the test-person sat up in order to be able to carry out work lasting 1 minute. This work being done, the resting position was again being resumed, and registration continued for 20 minutes.

The work was carried out on Krogh's cycle ergo-meter. As standard work, a load of 2 kg was chosen for women and 3 kg for men, this giving with 60 pedal revolutions, 780 and 1,170 kgm respectively. The rate was indicated by a metronome. It frequently proved difficult for the untrained test-persons to follow the rate indicated; each third revolution of the pedal was therefore indicated by the basis-pen and the work done calculated for each single case. All tests were carried out in a cellar room where an attempt was made to keep the temperature constant as far as possible.

In some respects it was seen that Scholander's apparatus cannot be used in these tests without a correction. Above all, this applies to the capacity of the cylinders. They each take 60 l and in order to achieve complete CO_2 absorption with subsequent filling of atmospheric air, 4 minutes are required, so that one had to change the cylinders every 4 minutes. The total CO_2 output in 4 minutes periods was therefore registered. In the working tests, however, the ventilation always exceeded 60 l in the first 4 minutes after work and now and then also in the next 4 minutes. In order to avoid the re-respiration of the expiration-air, one was therefore obliged to change the cylinder already after 2 minutes had passed, the CO_2 thus not being absorbable for these periods. A further consequence was that the CO_2 -excretion could neither be registered for the last period of rest, nor for the work-period, as the respective post-periods were too short.

The following changes were accordingly made (see fig. 1 a. b., p. 004) in Scholander's apparatus. A box with lamellae (*mixing-box*) of about 3 l was inserted on the expiration tube, in order to give an even mixture of the expiration-air. *Gas-recipients* were placed at the box outlet. During the periods when it was not possible to determine the CO_2 , continual expiration-air samples were taken and later ana-

lysed with the aid of Haldane's apparatus. The CO_2 -output could thus be calculated, as the ventilation was known. No regard was paid in the calculation to the amounts taken out (20—30 ml) by the ventilation measurement. During these periods the absorption lime and the CO_2 bell were uncoupled by the aid of an *auxiliary cock* (fig. 1 b) in order to ensure a complete exchange of expiration air for atmospheric air.

These changes made in Scholander's apparatus entail, however, also a few sources of error, the importance of which has been attempted to be shown by tests and calculations:

A small error in analysis when determining CO_2 % with Haldane's apparatus, represents comparatively large amounts of discharged CO_2 , assuming that ventilation is large. This was proved by the double analyses which were frequently (201 times in all) made with the expiration-air tests. The difference at each double-analyses (calculated in CO_2 at a ventilation of 100 l, which was not infrequent in the first 4 minutes after the work) gave the following values:

67.3 %	of 201 double analyses gave	0—50 ml CO_2
23.4 %	» » » » » »	50—100 » »
9.3 %	» » » » » »	100—300 » »

In double analysis, therefore, the middle value was used.

In three rest-tests (altogether 10 periods) the directly registered CO_2 amounts were compared with the values calculated according to the expiration-air analyses. A divergence of 0—70 ml CO_2 was found (average 28 ml CO_2) or 0—7.2 % of the discharged CO_2 amount according to direct registration. It is seen that the divergence would have been greater if double analyses had not been taken. Finally it was proved in four tests that after work ventilation falls, and with it the CO_2 , most in the first and second minutes, less in the third and fourth minutes of the first after-period. Three other tests showed that an uneven addition of expiration-air in the recipient in this period could give quite considerable difference in CO_2 %. In a test where it was arranged first for a rapid and then a slow current into one recipient and at the same time an even current in another recipient, the difference in CO_2 corresponded at a ventilation of 100 l to 250 ml CO_2 . In order to ensure a representative value for the CO_2 output it was thus necessary to aim at an even flow of expiration air to the recipient throughout the whole period. This frequently proved difficult and it represents a further source of error.

The Respiration Curves. Fig. 3. shows a section of a *respiration curve* (*RD-curve*) from a normal test-person registered on Scholander's apparatus. A detailed description of the curves, and a calculation of the values these represent, are given by Scholander (1937, fig. 27, p. 65).

On the *base-line* each 4 minutes period is marked off. As the pens which draw the ventilation and respiration curves are arranged vertically above and below the basis-line pen (see fig. 2)

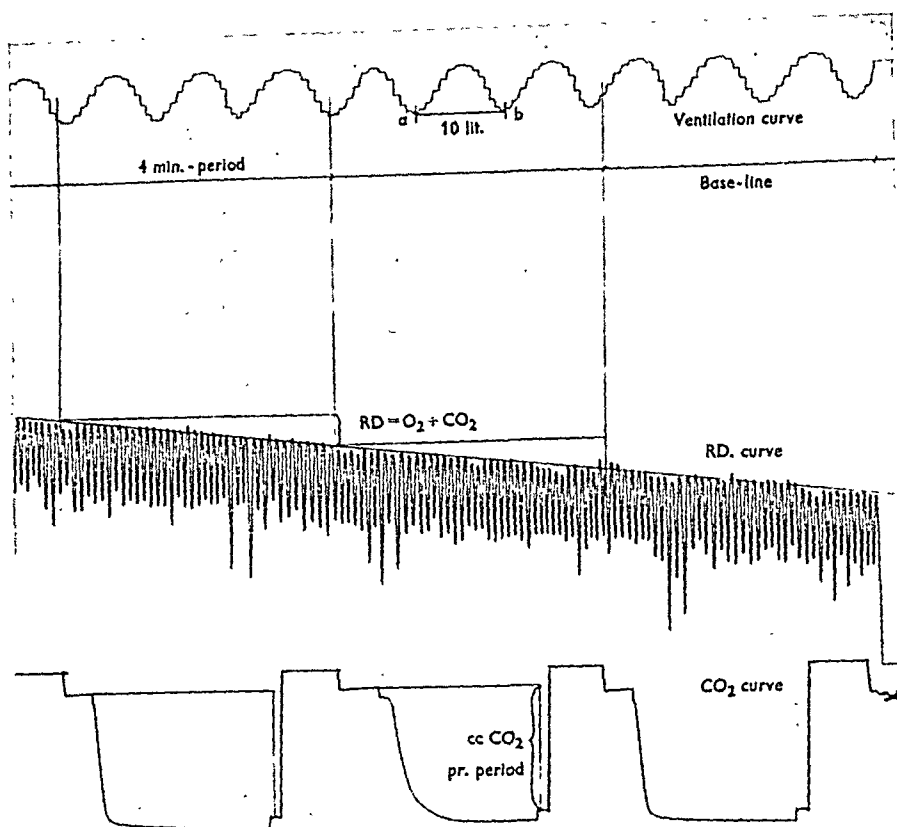


Fig. 3. Part of a respiration curve, showing the *base line* divided in 4 min. periods, transferable also to the other curves. Above it is seen the *ventilation curve* representing a series of 10 lit. airmasses, each corresponding to a revolution of the indicator wheel. Below the base line the *respiratory difference curve (RD.)* is drawn, illustrating the relation between O_2 acceptance and CO_2 output, and finally also the CO_2 curve illustrating the CO_2 output within each 4 min. period.

the division of the basis-line into 4 minutes periods by vertical lines, can also be transferred to these. From the CO_2 curve is calculated the CO_2 amount exchanged for each period. The distance a—b on the ventilation curve corresponds to a revolution of the wheel which represents 10 l. The *ventilation*, which henceforth always means the total of all inspirations in a given period, is calculated by counting the number of revolutions in the period. Interpolation is made where the lines of division fall between a—b.

A line drawn through the top points of the respiration curves indicates the respiratory difference (RD), *i. e.* the difference between O_2 acceptance and CO_2 output. When one thus knows

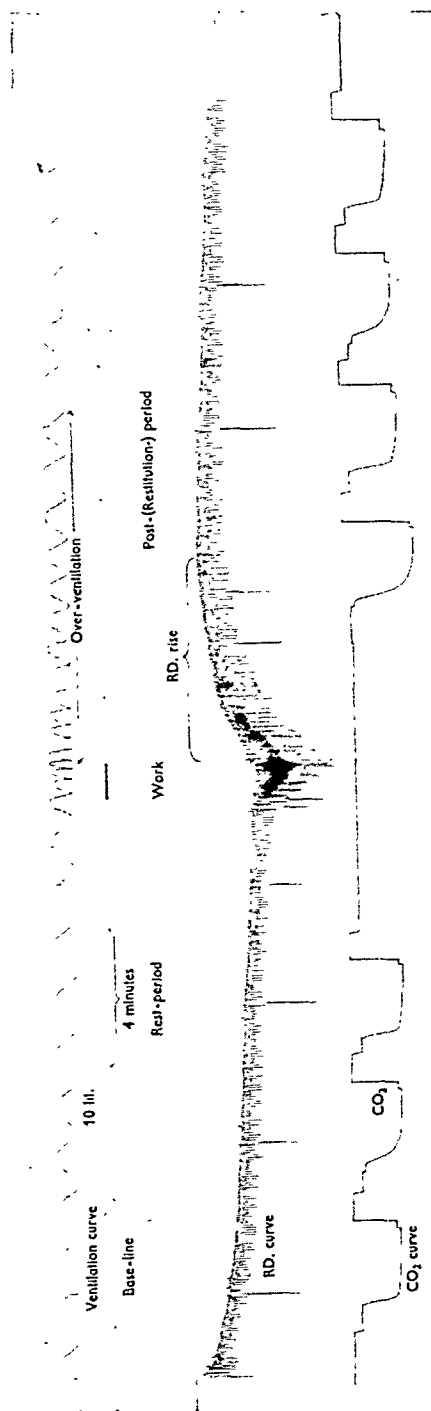


Fig. 4 a.

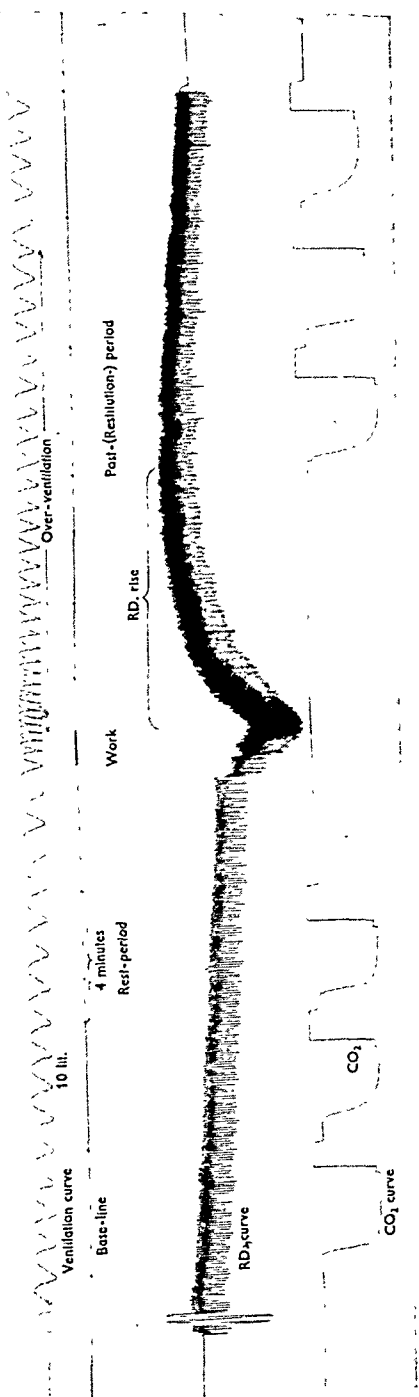


Fig. 4 b.

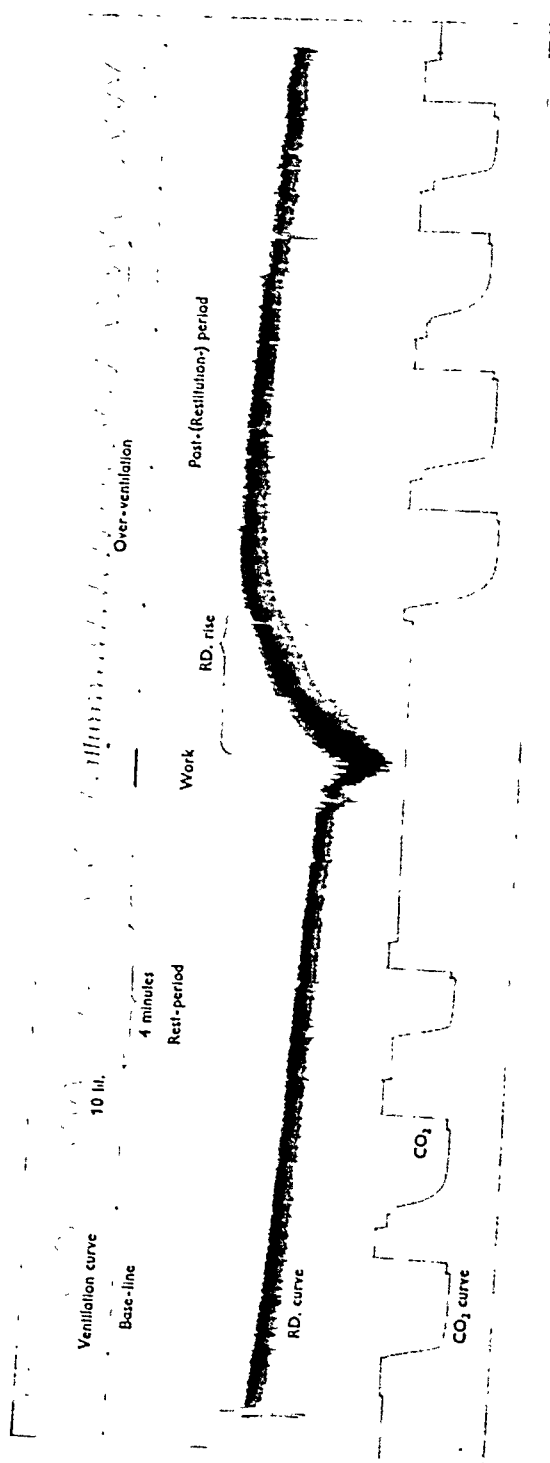


Fig. 4 c.

Fig. 4. Respiration curves from *a*: a normal woman (no. 290), *b* and *c* patients; ♂ (no. 304) and ♀ (no. 244). In *b* and *c* will be noted a higher frequency than in *a*. During the restitution (post-period) a higher and longer lasting rise of the RD-curve (RD.R.) may be seen as well as an augmented over-ventilation.

the discharged CO_2 amount and RD for each period, one can calculate the O_2 acceptance. $\text{CO}_2 + \text{RD} = \text{O}_2$. The respiration frequency can be read off direct on the curve by counting the number of inspirations in a given period of time.

The following values were also calculated:

Expiration volume = Inspiration volume \div RD

CO_2 % in expiration air = $\frac{\text{CO}_2 \text{ output} \times 100}{\text{Exp. volume}}$

O_2 % (accepted O_2 in % of insp. vol.) = $\frac{\text{O}_2 \text{ accept.} \times 100}{\text{insp. vol.}}$

Depth of respiration = $\frac{\text{ventilation}}{\text{frequency}}$

The volumes are measured at room temperature and without regard to the state of the barometer.

Respiration curves, as here described, were taken for each person examined, in all 40 normal persons (19 men and 21 women) and 159 patients (84 men and 75 women). Some of the curves are reproduced in fig. 4.

During the examination of this material, the following values were used:

1. For Rest.

CO_2 output per min in ml
Ventilation » » » litres
O_2 acceptance » » » ml
Respiration frequency in min.
» depth » litres.

All these values were calculated as the average of the 2nd and 3rd periods. The first and last periods were not considered, the first because some time elapsed before the test-person became accustomed to respiring in the apparatus, and the last, because during it some work was done preparatory to the working period.

2. For working period.

CO_2 over-output	in ml
Over-ventilation	» litres
O_2 over-acceptance	» ml
Respiration frequency	
Average respiration depth	» litres.

By over-ventilation, CO_2 over-discharge and O_2 over-acceptance is meant the respective values for the periods minus those of the rest-period. The respiration depth indicates the average for the period.

3. The post-(Restitution)-period.

CO₂ over-output in ml
 Over-ventilation » litres
 O₂ over-acceptance » ml
 Respiration frequency in the first minute after work.
 The average respiration depth in litres the first
 minute after work.
 The duration in minutes of the rise of the respira-
 tion difference curve (RD.R., fig. 4).
 The duration of the over-ventilation in minutes
 (Ov.V.).

RD.R. and Ov.V. were calculated from the cessation of work. The over-ventilation was calculated as increased for as long as the distance between the two apices on the ventilation curves was less than the average distance on the rest-curve.

The Application of the Method to the Material.

By means of the technique described, patients with various tuberculous lung manifestations and pleura affections, treated and untreated, were examined and compared with normal material tested in precisely the same manner. By thus comparing the normal average values with the corresponding values of all the patients examined, or for definite groups of these, we will be able to decide whether any general difference in respiration exists in the case of tubercular persons and normal, and where this divergence exists. To obtain a numerical basis for the comparison of the middle figure, Fischer's statistical methods (Fischer 1936, p. 166) for the evaluation of differences in small groups was used. If, in such a test, a difference of statistical importance is found between normal persons and patients, the conclusion may be drawn therefrom that the tubercular process has changed the function of the lung. Such a conclusion, however, would only be justified under the definite presumption that the groups to be compared are uniform with regard to the conditions which otherwise can have an influence upon respiration — something which during work with human beings offers considerable difficulty.

The greatest possible uniformity has been attempted above all by treating men and women in separate groups; thereafter, through a selection of normal material we have attempted to avoid major differences with regard to the average values for age, weight and height. Test conditions have been made as similar as possible

on all points. No one had been trained previously in the technique but most could cycle, and therefore had no difficulty in pedalling the ergo-meter. In one respect, however, there was considerable divergence between patients and normals: the normal test-persons were mainly nurses and functionaries at the sanatorium, going about their daily work, whereas the patients lived according to the usual regime of the sanatorium. The importance of this difference of condition for the results of the brief work-tests was difficult to determine, as we have not had the opportunity of testing this by precise investigations.

A factor to be taken into consideration in any comparison between patients and normal persons is the *individual variation* of the various respiratory values. In repeated investigations of the same test-person, these were frequently found to be far apart from each other (see p. 10) — so far as hardly to be explainable as a consequence of the small variations in the work done which easily might occur.

The importance of this individual variation, apparant in equally large degree in normal persons as well as patients, will, however, be highly reduced through the fact that not the absolute, but only the average values have been used in the comparison. Further, most test-persons as a rule were examined twice, sometimes several times, whereas only one curve from each individual was utilised for the statistical analyses. Thus, in the case of normal persons, the curve was always chosen which showed the highest reaction after work, while for patients, the curve showing the lowest reaction was chosen. The reaction was measured on the over-ventilation this being noted automatically without any intervention and thus burdened by the fewest possible methodical errors. Moreover, it was seen that the results were approximately the same whether CO_2 over-discharge after work was used, or O_2 over-acceptance during work was taken for the choice of curves. In cases where the difference in the amount of work in one and the same test-person was so great that it could be thought to have a bearing on the respiratory values, the curve was always chosen where the amount of work was nearest the standard work employed.

Statistical Treatment. The question of a difference in the respiration of normal persons and tuberculous, has been investigated according to Fischer's method which is particularly applicable for the treatment of small groups (Fischer 1936).

By this method an expression is found for the average group difference between the registered respiratory values in the case of normal persons and patients. If this difference (P) is not of »statistical importance», it is not permissible to conclude that the pathological changes in the patients have produced any change beyond the normal in their respiration. In order that such a difference shall be considered as being of statistical importance, it is required that the size P (*Probability*) < 0.01 , or in other words, that the chance that the difference is due casually is less than 1 in 100.

Own Investigations.

Normal Material.

The normal material employed consists of 21 women and 19 men. With this material altogether 83 tests were made in accordance with the method employed above.

For all respiratory values calculated on the basis of the curves the average was reckoned for all men and women. These average values are hereafter used as »normal figures». As »normal boundary values» is for each respiratory value given the *mean* (M) for the whole group ± 3 times the *standard divergence* (3σ).

All normal persons examined under similar test-conditions should be expected to give values lying within these normal limits, provided age and physical build are of the same order of size as in the individuals here examined. If therefore, in some cases values should be found which lie outside the normal limits, it will indicate a »respiratory insufficiency».

The age, weight, height and amount of work of the normal persons tested is seen from the following survey of our material:

	<i>Men.</i>	<i>Women.</i>
<i>Age:</i>		
15—19 years	2	2
20—24 »	4	1
25—29 »	4	9
30—34 »	3	5
35—39 »	5	4
40—44 »	0	
45—49 »	1	
<i>Weight:</i>		
60—64 kg	1	1
65—69 »	2	6
70—74 »	8	4

<i>Men.</i>		<i>Women.</i>	
<i>Weight:</i>			
75—79 kg	4	60—64 kg	6
80—84 »	1	65—69 »	3
85—89 »	3	70—74 »	1
<i>Height:</i>			
160—164 cm	1	145—149 cm	1
165—169 »	2	150—154 »	4
170—174 »	7	155—159 »	2
175—179 »	4	160—164 »	7
180—184 »	4	165—169 »	5
185—189 »	1	170—174 »	2
<i>Work done:</i>			
1050—1099 kgm....	2	600—649 kgm.....	1
1100—1149 »	1	650—699 »	4
1150—1199 »	14	700—749 »	4
1200—1249 »	2	750—799 »	7
		800—849 »	5

All our respiratory curves showed a very characteristic course. If we follow such a curve (Fig. 4) we find during rest an evenly falling respiratory difference curve (RD) as expressing that the oxygen acceptance is greater than the carbon dioxide output. The *respiratory quotient*, $RQ = CO_2/O_2$ is accordingly less than 1. During work the curve still falls ($RQ < 1$) but on the cessation of work it immediately commences to rise, this taking place in all curves registered. This increase means that the CO_2 output now is greater than the O_2 acceptance and RQ consequently greater than 1. The rise on the RD-curve lasted on an average about 6 minutes both for men and women reckoned from the cessation of work. When the maximum is reached the curve again resumes a falling course which continues throughout the rest of the test.

In Table I the normal mean values ($M \pm 3 \sigma$) are given.

It is seen from Table I that the CO_2 output, the ventilation and the O_2 acceptance increase during work. During restitution after work a CO_2 over-output and O_2 over-acceptance are similarly found, together with an over-ventilation (Ov.V.) which lasts, on an average, 7—8 minutes after work has ceased. There is little difference in the acceptance of O_2 during and after work, whilst there is a very great difference in the CO_2 output; in both cases the highest values are found after work, where the CO_2 over-output is very much greater than the O_2 over-acceptance. It is this excess-output of CO_2 compared to the O_2 acceptance which

Table I.

Mean respiratory values ($M \pm 3 \sigma$) of 40 normal individuals (19 men and 21 women).

See definitions on pp. 012—013.

	Rest-period		Working-period		Post-period	
	M ± 3 σ		M ± 3 σ		M ± 3 σ	
<i>Men:</i>						
CO ₂ output in ml. .	325	78	977	540	3205	1683
Ventilation in lit. . .	9.2	2.4	22.0	16.2	61.5	42.9
O ₂ accept in ml.	374	84	1538	657	1760	1329
Frequency	13.3	7.5	19.0	15.3	16.0	8.7
Depth. in lit.	0.711	0.366	1.655	0.924	2.104	0.726
<i>Women:</i>						
CO ₂ output in ml. . .	230	90	725	495	2544	1242
Ventilation in lit. . . .	7.6	3.6	18.0	17.7	48.5	34.5
O ₂ accept. in ml. . . .	268	78	1111	648	1408	813
Frequency	16.0	10.5	22.4	16.2	19.1	8.1
Depth in lit.	0.481	0.174	1.161	0.585	1.430	0.432
	RD.R.		Ov.V.			
	M ± 3 σ		M ± 3 σ			
Men:	6.4 min.	3.3	7.7 min.	5.3		
Women:	6.0 "	4.1	7.3 "	4.6		

produces the rise of the respiratory difference-curve (RD.R.) in the first minutes after work.

Patient-Material.

As mentioned above, the material of patients consists of 159 individuals (84 men and 75 women) having the most varied tubercular lung manifestations and pleura affections. The material has partly as a collective group been compared to the normal material, partly has the material of patients been divided into groups according to the changes revealed by the X-rays, each of these groups being compared with normal material. A number of patients were examined before as well as after collapse-treatment. The results of these examinations will be discussed in a special section.

Comparison Between the Total Patients and the Normals.

The average values for age, weight and height as well as of the work done are seen below:

	<i>Men.</i>		<i>Women.</i>	
	Patients	Normal	Patients	Normal
Age	28.2 years	29.4 years	26.6 years	28.8 years
Weight . . .	72.2 kg	74.3 kg	57.6 kg	58.2 kg
Height . . .	174.8 cm	174.3 cm	162.1 cm	160.8 cm
Work	1140 kgm	1162 kgm	726 kgm	744 kgm

As will be seen there is no essential difference in the average body size of the two groups and therefore this factor has not further been considered in the comparison. With regard to the amount of work done, this lies somewhat lower in the case of patients (22 kgm for men and 16 kgm for women). This is of significance in as much as it proves that an increase of the CO₂ output in the post-period must be due to changed respiration and not to a size difference.

The results of the statistical analyses may be seen in Tables II & III for men and women respectively. These tables indicate the difference between the mean values of the normal individuals and the patients. Negative values indicate that the patients' mean figures lie lower than those of normal individuals.

From the tables it can be seen that the patients during *rest* respire with a more rapid frequency. The depth of respiration is slightly lowered, but not enough for statistical certainty. There is no statistically ostensible change in the CO₂ output pr minute. For both men and women a hyper-ventilation is found which shows itself to be of definite statistical importance ($P < 0.01$). In the case of men, the O₂ acceptance is also lowered, seen statistically, but no change can be proved for women.

During *work* the difference between normal individuals and patients increases with regard to frequency as well as depth and for both the divergence is statistically important. Further, it will be noticed that the CO₂ over-output and the over-ventilation during work are practically the same for patients and normal persons, whereas the O₂ over-acceptance in patients is statistically proved to be less than in normals.

Also in the first minute of the *after-period*, the patients' frequency is increased and the depth lowered, with statistical cer-

Table II.

Statistical analysis of respiratory values showing the difference between the mean values of male patients and those of normal men. All differences of statistical importance ($P < 0.01$) are marked with heavy types.

Men	No.	Frequency pr. min.			Depth lit. pr. min			CO ₂ output ml pr. min.			Ventilation lit. pr. min.			O ₂ acceptance ml pr. min.				
		N + 1	M ±	3 σ	V %	M ±	3 σ	V %	M ±	3 σ	V %	M ±	3 σ	V %	M ±	3 σ	V %	
Rest-period	Patients...	76	16.5	11.1	22.55	0.647	0.42	21.8	313	105	11.31	10.3	4.5	14.75	346	111	10.58	
	Normals ..	19	13.3	7.5	19.02	0.711	0.36	17.16	325	78	8.06	9.2	2.4	8.85	374	84	7.49	
	Difference. Probability		3.2	$P < 0.001$		÷0.064 $P = 0.10$			÷12 $P = 0.20$			1.1 $P < 0.01$			÷28 $P < 0.01$			
Working- period									CO ₂ over-output			Over-ventilation			O ₂ over-acceptance			
	Patients...	76	24.5	18.3	24.7	1.412	0.88	20.80	961	621	21.55	23.1	16.5	23.65	1273	642	16.80	
	Normals ..	19	19.0	15.3	27.0	1.655	0.92	18.62	997	540	18.05	22.0	16.2	24.30	1538	657	14.24	
	Difference. Probability		5.5	$P < 0.001$		÷0.243 $P < 0.01$			÷36 $P = 0.5$			1.1 $P = 0.4$			÷265 $P < 0.001$			
1 st. min.	Patients ..	76	23.3	15.3	21.60	1.701	1.01	19.75										
	Normals ..	19	16.0	8.7	18.30	2.104	0.73	11.48										
	Difference. Probability		7.3	$P < 0.001$		÷0.403 $P < 0.001$												
	Patients ..	76	8.0	5.1	20.60	11.1	10.8	32.40	4315	2448	18.95	94.5	64.8	22.90	2269	1626	23.85	
	Normals ..	19	6.4	3.3	17.10	7.7	5.3	23.10	3205	1683	17.51	61.5	42.9	23.25	1760	1320	25.00	
Post-period	Difference. Probability		1.6	$P < 0.001$		3.4 $P < 0.001$			1110 $P < 0.001$			33.0 $P < 0.001$			509 $P < 0.001$			
			Rise of resp. curve			Over-ventilation												
		Duration in min.																

* Frequency and depth registered in 18 Normals

Table III.
Statistical analysis of respiratory values showing the difference between the mean values of women patients and those of normal women. All differences of statistical importance ($P < 0.01$) are marked with heavy types.

those of normal women and men.

Women	No.	Frequency pr. min.			Depth lit. pr. min.			CO ₂ output ml pr. min.			Ventilation lit. pr. min.			O ₂ acceptance ml pr. min.				
		N +1	M ±	3 σ	V %	M ±	3 σ	V %	M ±	3 σ	V %	M ±	3 σ	V %	M ±	3 σ	V %	
Rest-period	Patients ..	67	19.2	15.3	26.50	0.457	0.222	16.20	241	93	12.80	8.6	4.8	18.00	279	87	10.53	
	Normals ..	21	16.0	10.5	21.83	0.481	0.174	12.05	230	90	13.05	7.6	3.6	15.65	268	78	9.70	
	Difference. Probability		3.2 P=0.91			÷0.024 P=0.2			11 P=0.15			1.0 P<0.91			11 P=0.1			
Working-period	Patients ..	67	29.7	21.0	23.40	0.959	0.66	22.80	694	510	24.50	19.1	12.9	22.40	889	618	23.20	
	Normals ..	21	22.4	16.2	24.10	1.164	0.59	16.75	725	495	22.75	18.0	17.7	32.85	1111	648	19.40	
	Difference. Probability		7.3 P<0.901			÷0.205 P<0.901			÷31 P=0.5			1.1 P=0.4			÷222 P<0.901			
1 st. min.	Patients ..		26.6	15.6	19.62	1.178	0.57	16.22										
	Normals ..		19.1	8.1	14.10	1.430	0.43	10.07										
	Difference. Probability		7.5 P<0.901			÷0.252 P<0.901												
Post-period	Patients ..		7.4	5.4	24.90	11.3	10.2	30.00	3023	1875	20.65	72.7	53.1	24.25	1524	1206	26.40	
	Normals ..		6.0	4.1	22.40	7.3	4.6	20.80	2544	1242	16.28	48.5	34.8	24.05	1408	813	19.23	
	Difference. Probability		1.4 P<0.91			4.0 P<0.901			479 P<0.91			24.2 P<0.001			116 P=0.2			
		Rise of resp. curve			Over-ventilation													
		Duration in min.																

¹ Frequency and depth registered in 66 patients.

tainty in both cases. The total CO_2 over-output and the over-ventilation during the entire post-period is greatly increased in the patient, and the CO_2 over-output much more than the O_2 over-acceptance, the increase of which is statistically certain for men only.

The duration of the rise in the respiratory difference-curve is extended for patients; likewise the duration of the over-ventilation, with statistical certainty in both cases.

Comparison Between the Separate Clinical Groups and Normal Material.

In order to investigate the influence of the different lung manifestations on the respiration, the clinical material is divided into the following groups, for both men and women:

- Group 1. slightly spread parenchym changes.
- Group 2. developed one- or two-sided parenchym changes.
- Group 3. slight one-sided pneumo-thorax.
- Group 4. well-developed one-sided pneumo-thorax.
- Group 5. double-sided pneumo-thorax.
- Group 6. pleurisies.
- Group 7. one-sided thoraco-plastic with apicolysis.
- Group 8. one-sided thoraco-plastic with apicolysis, and parenchym changes on the other side.

Each of these groups has been compared with the normal material. The clinical division had been made by Dr. Gjessing according to the lung changes shown by the X-rays, before the results of the respiration investigations were available. It was impossible to obtain entirely uniform groups and therefore there is little marked difference between »slightly spread» and »spread» parenchym changes; nor is there any distinct difference among the pneumo-thoric groups, where also are to be found more or less extensive parenchym changes. In the pleurisy groups old pleurisies (adhesions and thickening of the pleura) and pleurisy with effusion are found, just as in the clinical divisions no regard has been paid to the movability of the diaphragm or to the spreading of the adherences in pneumo-thorax.

As the number of patients in each group is small, the certainty of the statistical results is reduced. The analysis errors mentioned above and variations-casually appearing in the case of any single patient may here give rise to disproportionately large changes in

the average group figure. Fischer's statistical methods, which have been used here, however, are just calculated with the view to comparing small groups, and lay special stress on taking into consideration the spreading from the mean figures.

Tables IV and V show the results of the statistical analyses for all groups. The figures given here show, as in Tables II & III the difference between the normal mean values and the mean values of each single group of patients. The minus sign indicates that the patients' mean value is less than that of the normal person. Figures in heavy type indicate $P < 0.01$, thus giving complete statistical certainty that the divergence is real. The difference which gives values for $0.01 < P < 0.02$ are in italics. Even these values can be given a certain significance, although they do not give full security for the divergence between normal persons and patients being real.

Table IV reproduces the results of such a comparison in the case of men.

During *rest*-respiration there is found, as it will be seen, a statistically traceable hyper-ventilation for the groups 1, 4 and 6 and an increased O_2 -acceptance for group 5. The frequency for groups 4, 6 and 7 is also increased. Otherwise, there is no characteristic difference between the various groups.

During *work* there is no statistically traceable difference from the normal figures, either for CO_2 -secretion or for over-ventilation, whereas for all groups there is less O_2 over-acceptance than normal. This lowered oxygen over-acceptance is statistically certain for groups 2, 4, 5, 7 and 8, and of some importance for group 3. The frequency is increased for four of the groups, viz. 4, 5, 6 and 7. The respiration depth is lowered for groups 4 and 5.

In the *after*-period an increased CO_2 over-secretion is found for all groups; similarly, over-ventilation is increased for all groups. For group 3, although the increase in over-ventilation is certainly not statistically certain, it is of some importance. For groups 4, 6 and 7 an increased O_2 -over-acceptance is found and for groups 2, 5 and 8 an increase of some importance. Frequency in the first minute after work shows an increase in all groups, statistically traceable for all groups except no. 1. The respiration depth is lowered except for groups 1 and 2. The rise in the respiratory difference curve is definitely extended for groups 2, 3, 4, 5 and 7 and approaches certainty also in group 8. The duration of over-ventilation after work is extended for groups 2, 4, 5, 7 and 8 and somewhat extended for group 3.

There is thus found within the separate groups of *male* patients as in the treatment of the whole material, that a lowered oxygen acceptance during brief work is accompanied by an increased

Table IV.

Results of statistical analyses for separate clinical groups of male patients, showing their difference from the values of normal men. Figures in heavy types, indicating $P < 0.01$, give values of statistical importance, while difference values for $0.01 < P < 0.02$, having a certain statistical significance, are in italics.

Group		Rest					Work-period					Post-period			1 min. after work		RD.R.	Ov.V.
No.	Ind.	CO ₂	Vent.	O ₂	Fr.	D.	CO ₂	Vent.	O ₂	Fr.	D.	CO ₂	Vent.	O ₂	Fr.	D.		
1	7	10	1.4	4	2.4	÷ 0.075	÷ 150	÷ 1.9	÷ 138	1.0	÷ 0.075	731	19.6	141	3.6	÷ 0.084	1.3	0.7
2	10	÷ 7	1.1	÷ 9	3	÷ 0.054	4	÷ 0.2	÷ 268	3.6	÷ 0.135	1100	38.2	483	5.6	÷ 0.218	1.7	4.3
3	13	÷ 7	0.8	÷ 12	2.1	÷ 0.024	÷ 47	÷ 0.3	÷ 187	3.6	÷ 0.156	1146	29.5	386	6	÷ 0.334	1.9	2.3
4	10	÷ 10	1.7	÷ 19	4.6	÷ 0.107	÷ 36	2.3	÷ 313	8.8	÷ 0.363	1123	33.2	587	9.6	÷ 0.521	1.4	3.2
5	4	÷ 41	0.4	53	0.5	0.023	÷ 184	1.0	÷ 441	9	÷ 0.556	1686	56	698	9	÷ 0.639	4.1	10.1
6	9	1	1.8	9	4.1	÷ 0.085	52	4.2	÷ 209	6	÷ 0.171	1260	29.1	753	7	÷ 0.366	1	1.6
7	6	÷ 30	0.8	÷ 36	4.9	÷ 0.140	12	2.6	÷ 356	8	÷ 0.373	1179	34.1	692	8	÷ 0.516	1.6	4.5
8	8	÷ 23	0.7	÷ 23	2.5	÷ 0.067	÷ 52	0.6	÷ 327	5.6	÷ 0.371	1105	39.1	466	10	÷ 0.569	1.2	4.3

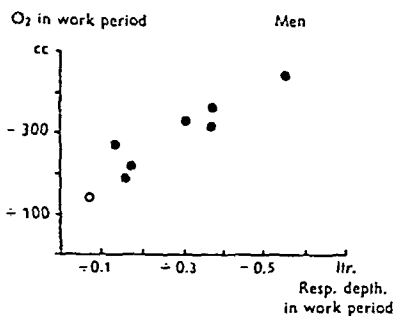


Fig. 5 a.

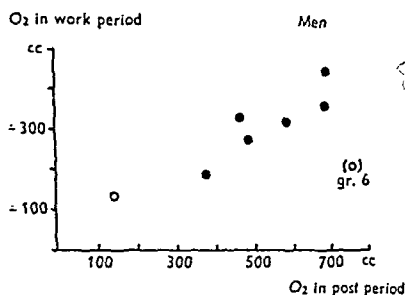


Fig. 5 b.

Fig. 5. Respiration values in clinical groups of male patients. (Cfr. Tab. IV) • means value of statistical importance.

a: Correlation between O₂ acceptance and respiration depth during work.

b: Correlation between O₂ acceptance during work and in the post-period.

CO₂ output and ventilation after work, together with an extension of the respiratory difference curve and also of the duration of the over-ventilation.

It seems, according to clinical judgement, that the depth of the respiration is dependent upon the lungs' respiration-capable tissue. Where the depth is lowered during work, it is lowered still more after work. The reduction of respiration depth is seen to be nearly proportional to the decrease of O₂ over-acceptance in the work period (see fig. 5 a). Similarly, it seems that the O₂ over-acceptance after work is approximately proportional to the decrease during work, which is apparent from fig. 5 b. The pleurisy group here forms an exception as, notwithstanding that there is no statistically traceable decrease in the O₂ over-acceptance during work, there is nevertheless found a greater O₂ over-acceptance after work than in any of the other groups.

Over-ventilation after work varies within certain limits parallel with the CO₂ over-output, and the duration of the rise in the resp. diff. curve often seems, after work, to increase with rising CO₂ over-output; similarly, the duration of over-ventilation increases with the extent of the ventilation (Tab. IV).

It was found during the treatment of the complete female material (see p. 018—020) that, as with the males, there was on the average a lowering of oxygen acceptance during brief work and an increased carbon dioxide output as well as an over-ventilation after work, together with an extension of the rise in the respiratory difference curve and the duration of over-ventilation; further,

Table V.

Results of statistical analyses for separate clinical groups of female patients, showing their difference from the values of normal women. Figures in heavy types, indicating $P < 0.01$ give values of statistical importance, while difference values for $0.01 < P < 0.02$, having a certain statistical significance, are in italics.

Group		Rest					Work-period					Post-Period			1 min. after work		RD.R.	Ov.V.
No.	Ind.	CO ₂	Vent.	O ₂	Fr.	D.	CO ₂	Vent.	O ₂	Fr.	D.	CO ₂	Vent.	O ₂	Fr.	D.		
1	9	4	0.3	8	1.2	÷ 0.007	74	1.4	÷ 89	÷ 0.3	0.107	721	22.1	312	4.3	÷ 0.083	0.6	3.8
2	11	20	1.2	14	2.9	÷ 0.008	54	0.0	÷ 225	7.1	÷ 0.214	294	18.9	÷ 45	7.2	÷ 0.199	1.5	2.2
3	10	13	0.6	10	2.2	÷ 0.023	81	4.0	÷ 80	7.2	÷ 0.118	502	27.5	37	5.6	÷ 0.239	2.9	5.2
4	8	22	1.5	24	5.5	÷ 0.022	÷ 104	÷ 1.0	÷ 251	8.2	÷ 0.270	130	15.5	÷ 146	9.5	÷ 0.332	0.9	1.6
5	7	÷ 14	0.0	÷ 15	0.9	÷ 0.022	÷ 127	÷ 1.0	÷ 356	6.6	÷ 0.298	672	25.5	229	4.9	÷ 0.314	2.0	5.8
6	6	15	1.4	12	5.4	÷ 0.048	÷ 18	3.5	÷ 137	9.9	÷ 0.232	335	15.0	246	6.7	÷ 0.221	0.7	3.9
7	5	9	0.8	17	4.4	÷ 0.060	÷ 88	÷ 0.5	÷ 338	10.6	÷ 0.311	676	31.8	295	10.6	÷ 0.387	2.0	5.7
8	7	10	0.8	14	3.4	÷ 0.039	÷ 66	1.0	÷ 395	11.2	÷ 0.311	216	26.1	26	11.9	÷ 0.353	0.9	4.5

it was similarly found that the frequency and the depth of respiration during and after work increased and decreased respectively.

Closer examination of the separate groups shows, however, that men and women differ at certain points, even if a direct comparison of the average values between the male and female groups cannot be carried out, owing to — among other reasons — the difference in the work done, by the latter.

Table V gives the results of a comparison between the clinical female groups and the normal material.

During the *rest-period* no changes in CO_2 output, O_2 acceptance and respiration depth were found. Apart from group 4, which shows an increase, ventilation is unchanged. The frequency is probably increased in groups 4 and 6, otherwise unchanged.

During *work* there is no statistically traceable divergence from the normal material either for CO_2 over-output or for over-ventilation. The O_2 over-acceptance on the other hand, is statistically definitely lowered for groups 4, 5, 7 and 8, and probably, for group 2 as well; frequency has certainly increased for all groups excepted 1 and 5, the latter group showing, however, also an increase of some significance. It is statistically certain that the respiration depth is lowered for groups 2, 4, 5, 7 and 8 and with some significance for group 6.

In the *after-period*, a statistically traceable increase of CO_2 over-output is found for groups 1, 3, 5 and 7. The over-ventilation is increased for all groups, the increase for group 6, however, not being statistically certain. There is no statistically traceable increase of the O_2 acceptance. The frequency is increased for all groups and depths decreased for all, except group 1. The rise of the respiratory difference curve after work is extended for groups 2, 3 and 5. Over-ventilation is extended for all groups; in group 4, however, the extension is not statistically certain.

During work there is thus found in the case of women, similar conditions as with men. Corresponding to increasing extension of parenchym-changes or increasing collapse, clinically judged, an increased lowering of the respiration depth and oxygen acceptance is found. Here again, in the case of women, proportionality seems to exist between the lowering of respiration depth and oxygen acceptance (see fig. 6 a). Group 1 is an exception where the divergence from normal figures is without statistical significance.

The CO_2 over-output in women as in men shows an increase after work, notwithstanding that in the various female groups (1 and 3) no definite decrease of O_2 acceptance during work has appeared. Reversely, in the case of two of the female groups (4 and 8), there is a statistically traceable lowering of O_2 over-acceptance during work without any definite increase of CO_2

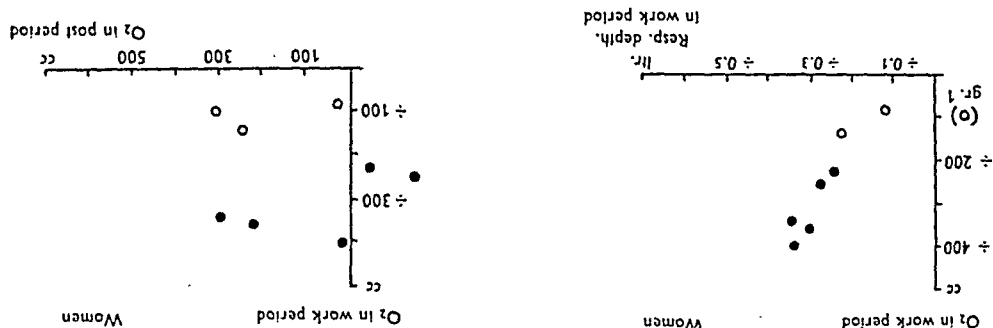


Fig. 6. *Respiration values in clinical groups of female patients.* (Cfr. Tab. V) • means values of statistical importance.

a: Correlation between O_2 acceptance and respiration depth during work.
b: Correlation between O_2 acceptance during work and in the post-period.

over-output after work, a condition which has not been found with men. In all cases where the O_2 over-acceptance during work was lowered, a statistically definite increase of CO_2 over-output in the after-period was here found. Similarly, in the case of women, there does not seem to be any agreement between O_2 over-acceptance during and after work (see fig. 6 b) as was the case with men. With regard to the rise of the respiratory difference curve (R.D.R.) and the duration of the over-ventilation (O.V.) the conditions found in both men and women are similar. Group 1, however, is an exception where a larger CO_2 over-output after work is found than in any of the other groups without extending the rise of the curve. Whether or not the divergences mentioned here between men and women in our material have any real importance, cannot, for the time being, be decided.

Respiratory Insufficiency.

By «respiratory insufficiency» is as previously mentioned here meant that an individual examined with the same technique as the normal material, lies outside the variation limits of the normal material in one or more of the respiratory values. We shall discuss briefly below, how often «respiratory insufficiency» was found among the patients treated in the clinical groups and what values lay most frequently outside the extent of normal variation.

For each of the respiratory values examined, the extent of normal variation for men and women has been stated separately

with the average values (M) for the whole group ± 3 times the standard value (3σ). Spreading in the normal material is great, and in the case of many patients respiratory values are found lying on the border of the normal extent of variation. The transition from »normal» values to values expressing »respiratory insufficiency» is therefore gradual, and the difference between these values is often insignificant.

In Table VI is also given, for each of the clinical groups, besides the total number of patients examined, also the number of individuals showing »respiratory insufficiency» for one or several values. It is seen from the table that »respiratory insufficiency» was found in 45 of the 67 male patients examined, and in 39 of the 63 female patients for one or several of the respiratory values. The material in the clinical groups is too small to give any definite impression as to whether within each of them any correlation is found between the number of patients with »respiratory insufficiency» and the clinical lung changes which characterise each one of them.

During the *rest-period* »respiratory insufficiency» is found only in few cases. In the case of male patients, it is the minute-ventilation which is most frequently increased beyond normal limits (in 13 cases); thereafter comes the frequency per minute (increased in 7 patients). The carbon dioxide output and the oxygen acceptance per minute shows each an increase in three cases only. — In women, during rest, »respiratory insufficiency» appeared even more rarely than in the case of men. Minute-ventilation and frequency per minute were found increased beyond the limits of the normal material in 4 and 5 cases respectively, the carbon dioxide output per minute was increased in the case of one patient only, and the oxygen acceptance per minute in two cases.

Investigations during brief *work* showed that most values for both men and women were within the limits of the normal material. Notwithstanding that the average oxygen-acceptance was, as previously mentioned, decreased statistically for several of the groups, the oxygen-acceptance was found in one male patient only, to be below the calculated lower limit for normal material. It was the frequency only which in 4 men and 9 women gave values higher than the upper limits of the normal material.

It is further apparent from the table that it is during the *post-period* after the brief work that a »respiratory insufficiency» most often is found in patients. In the case of men, it was the frequency in the first minute after work which was most often increased beyond the upper limits of the normal material (in 19 cases). Over-ventilation and CO_2 over-output were increased in 17 and 15 cases respectively. The duration of over-ventilation was extended beyond the normal in

Table VI.
Number of individuals within each of the clinical groups (men and women separately) showing respiratory insufficiency for one or several values.

insufficiency for one or several

Group		Patients with respir. insuff.	Rest-period				Working-period				Post-period				1st. min. after work		RD.R.	Oc.V.	
No.	Ind.		CO ₂	Vent.	O ₂	Fr.	D.	CO ₂	Vent.	O ₂	Fr.	D.	CO ₂	Vent.	O ₂	Fr.			D.
Men																			
1	7	2	1	1	1	1							2	3	1	2	1	3	
2	10	7	1	2	1	2							4	3	1	3	2	2	
3	13	7		3		1					2		2	3	1	5	4	1	
4	10	9		3		1					1		2	2	2	1	1	2	
5	4	3	1										2	2	1	2	2		
6	9	7		3		1							3	2	1	2		1	
7	6	3	1	1	1	1							1	1	1	2	3	2	
8	8	7			1					1			2	3		1		1	
Sum:	67	45	3	13	3	7	0	0	0	1	3	0	15	17	5	19	12	9	13
Women																			
1	9	5											2	2	1	1	1	3	
2	11	6	1	2	1	2					1		1	3		4	1	2	
3	10	7									1		1	4		3	1	6	
4	8	3				1					2					3	3		
5	7	5									1			1		1	1	1	
6	6	3		1		1					1		1	1	1	1		3	
7	5	4		1	1	1					2		1	2		3	2	1	
8	7	6									1			1		1	3	1	
Sum:	63	39	1	4	2	5	0	0	0	0	9	0	6	11	2	20	11	3	25

13 patients, and the duration of the rise of the respiratory difference curve (RD.R.) in 9. The respiration depth was in 12 cases less than the extreme limit for the normal material, whereas the O_2 over-acceptance was increased in 5 cases only beyond the variation breadth of the normal material. — In the case of women, »respiratory insufficiency» was found most frequently with regard to the duration of over-ventilation (increased in 25 cases). Thereafter, it was the frequency in the first minute after work which was in 20 cases increased beyond the limits of the normal material. Over-ventilation and CO_2 over-output gave, in 14 and 6 cases respectively, values which were larger than the upper limits of the normal material. In 11 patients the respiration depth was decreased, in 3 the rise in the respiratory difference curve was extended, while in 2 cases only the O_2 over-acceptance was greater than the upper limit of the normal material.

It is apparent from the above that not one of the respiratory values examined was outside the variation breadth in *all* cases where »respiratory insufficiency» was found. In order to prove the existence of the latter it would therefore not be sufficient to examine merely a single one of the respiratory values; further, it was stated that it was during the after-period these values most frequently exceeded the limits of the normal material and that, primarily, the »respiratory insufficiency» found expression through the frequency in the first minute, over-ventilation, carbon dioxide over-output and also in the duration of the over-ventilation. With the oxygen over-acceptance, however, we found altogether only 7 patients, the values of which were greater than the statistically calculated upper limit in the normal material.

Examination of Patients before and after Collapse Treatment.

In order, if possible, to discover the effect pneumo-thorax or thoraco-plastic has on the respiration, we examined 16 patients (8 men and 8 women), 2 of whom were subjected to pneumo-thorax and 14 to thoraco-plastic with apicolysis. We then worked out the differences for the various respiratory values.

These differences were then compared with the *maximum individual variation*. The variation was found in the following manner: The differences of all the respiratory values examined were worked out for all test-persons (normal and patients), who were examined twice or several times, and the differences (in all 401) were then divided into groups, regard being paid to the amount of work performed. If the differences before and after the collapse treatment as a rule should prove to exceed the individual variation, one would be permitted to conclude that a treatment like plastic or pneumo-thorax has had an influence on the respiration.

It was found, however, that the individual variation is very high. It may be mentioned for example, that one patient (H. L.) before collapse-treatment had a CO_2 over-output in the post-period of 3,795 ml and after the treatment 3,282 ml, one of the greatest differences, 513 ml, whereas one normal person (E. A.) in three examinations shows differences of 632 ml, 832 ml and 200 ml.

This result and the fact that the values of variation appeared so scattered and irregular, has proved that these investigations did not allow any definite conclusions.

Discussion.

We now turn to a discussion of the value of the above statistical results. In accordance with Fischer's indications, regard is only paid to those differences which give $P < 0.01$. The possibility is also considered that the changes in the patients' respiration could be produced by the casual coincidence of analysis inaccuracies and individual variations. It has been explained (see p. 9) how we have attempted to reduce the importance of the individual variation. With regard to analysis errors, however, one might presume that the importance of these is reduced by calculating with the average of numerous tests, just as they are partially eliminated by continually reckoning with the differences between two groups where the analysis errors make themselves evident in the same degree. We therefore consider it permissible to consider the differences found between normals and patients as an expression of clinical changes in patients, well knowing, however, that other causes also may be co-determinating in the divergences found in the respiration of patients (*e. g.* the better condition of normal persons than of patients).

Owing to the pathological changes in the lungs, the tissue capable of respiration is restricted. Sonne (1936) has shown that a normal lung cannot be ventilated with equal effectivity in all sections. One must consequently presume that a pathological process which restricts the tissue capable of respiration is not everywhere of the same importance for the respiration but that it may be dependent upon the localisation of the process. It appears, however, in the treatment of the clinical groups in our material that, on the whole, there is agreement between the pulmonary changes and the respiratory results. Thus, as previously mentioned, the restriction in the depth of respiration increases during and after work with the spreading of parenchym changes and the extent of the collapses. Similarly, correlation exists between the restric-

tion of the depth of respiration and the oxygen over-acceptance during work so that the oxygen acceptance decreases, the more the depth of respiration is lowered. As a consequence of the decreased respiration depth, the patients must, when demands rise, increase the ventilation of the lungs by respiring with a more rapid frequency than normal, the result being a superficial and little appropriate respiration, with decreased utilisation of the ventilation.

As is already mentioned, the patients take in less oxygen during the brief work period than the normal test-persons, whereas the carbon dioxide output and the ventilation are approximately the same for patients and normal persons. The Knipping school has shown that patients with similar pulmonar changes as in our material, during »steady state» may often take in more oxygen per minute than what was usual during work in our investigations. There is, therefore, but slight probability that in our tests the limit of maximum oxygen acceptance by patients has been exceeded, and that the oxygen acceptance for that reason is less than in normal persons. It is thus probable that the decreased oxygen acceptance during brief work, besides upon the pulmonal changes may also be dependent upon the regulatory processes which take place during the commencement of work, before the organism is able to adapt its supply of oxygen to the work to be done. The importance of the method as a single lung-function test is therefore reduced even if, generally spoken, an agreement was found between certain clinical changes and the respiratory results.

As already known, the first period of muscular work is anaerobic and changes when the body has adapted itself to the increased demand. During the anaerobe work lactic acid forms in the muscles of which a part is removed oxydatively on the spot, while another part goes into the blood. The lactic acid concentration in the blood thus depends upon lactic acid production and the oxydative removal. The lactic acid that goes into the blood forces carbonic acid out from the plasma's sodium bicarbonate and the carbonic acid is expelled through the lungs as carbon dioxide and produces the previously mentioned rise in the respiration curve. Scholander (1940) has, in seals after diving, proved a rise to occur in the respiratory difference curve similar to that seen in our curves. At the same time he found in the blood a rise in the lactic acid that was proportional to the respiratory difference and to the fall of carbon dioxide in

the blood. He could thus approximately determine the maximum rise of the lactic acid in the blood.

If then, the same conditions apply in our investigations, one should thus in the rise of the respiratory difference curve, or in other words, in the surplus carbon dioxide output after work, approximately obtain an indicator of the anaerobic work, which again is dependent upon the degree of lack of oxygen in the muscles, without requiring to produce lactic acid tests in the blood. To verify this, a series of tests were commenced where one, at the same time, registered respiration curves and undertook lactic acid analyses of the blood. Owing to the author's death, these investigations were not, however, carried far enough to enable conclusions to be drawn from the results. There are, however, other conditions which indicate that there may be some connection between the lactic acid concentration in the blood and the rise of the respiratory curves. Bang (1935) showed *inter alia* that during brief work the lactic acid concentration in the blood was rising for about 7—8 minutes, reckoned from the commencement of the work — an observation which agrees very well with the respiratory difference curve having in normals reached its maximum on an average about 7 minutes, reckoned from the commencement of the work.

The fact that the patient takes in less oxygen during the brief work than the normal test-person, is probably an expression of the oxygen supply to the working muscles being less than normal and the anaerobic work correspondingly greater. Considering that a possible change in the anaerobic work is reflected in the carbon dioxide over-output one would expect to find in the post-period of patients an increased over-output of carbon dioxide. The statistical analyses show that this is actually the case. Similarly, the rise of the respiratory difference curve is extended, which may indicate that the lactic acid rise in the blood is also extended.

There are two factors, which besides the anatomical changes in the lungs and the regulatory processes, may be supposed to be co-operating in determining the divergences which may be found among patients, viz. a) training and b) hyper-ventilation.

a) Bang (1935) has shown that in work-tests training plays a large rôle in the rise of the lactic acid in the blood. The rise is less with the trained than with the untrained. In our material *all* our test-persons were untrained in the technique employed. The difference in the condition in the two groups can, however, be supposed to play a rôle, but to a certain extent this may be con-

tradicted by the fact that, as previously mentioned, agreement may be found between the clinical changes and the respiratory findings.

b) Bang states further (*loc. cit.*) that untrained test-persons show a tendency to hyper-ventilation but that this hyper-ventilation within the limits of the work-tests does not entail marked changes in the lactic acid concentration in the blood. This is different, in our investigations where the shape of the respiratory curve partly depends on the CO_2 output which increases during hyper-ventilation. It is seen that during work there is no noticeable difference in the ventilation of normal persons and patients, and that one may also here presume that a possible hyper-ventilation will make itself felt in somewhat the same degree in all of them. This source of error will therefore also be eliminated through the fact that it is the difference between the two groups which form the basis of the calculation.

Bluhm (1935) indicates that tuberculous persons after brief work show a larger «oxy indebt» than normal persons and that the increase in «oxy indebt» exhibits good agreement with the pulmonary changes. By «oxy indebt» is understood the oxygen-intake after work calculated in percentage of the values in the rest-period. As already pointed out (see p. 021) in our material it is only the male patients who show a statistically definite increase of oxygen-intake after work. The oxygen deficit which arises during work is covered during the restitution period after the work as proportionality exists between the oxygen deficiency during work and the oxygen over-acceptance after work. The fact that the oxygen over-acceptance after work is even higher than the oxygen deficiency during work indicates that it is not merely a simple covering of the oxygen deficiency that takes place but that at the commencement of the work complicated processes occur which, with our methodics, have not yet been understood. In the pleurisy group one finds a statistically increased oxygen over-acceptance after work, without there being any statistically traceable oxygen deficit during work (Tab. IV, group 6). To what extent this condition rests upon something casual or is to be explained by the reaction of the organism owing to the methodics employed, cannot be determined because of the small number of group individuals examined.

The results of the present investigations will with regard to the respiration of tuberculous persons burdened by a short transitory work, indicate the following conclusion:

As a consequence of the anatomical changes in the tuberculous lung, there arises during brief work an oxygen deficiency that entails changes in the anaerobic work of the muscles. These changes find expression in the gaseous exchange in the lungs which have been followed continuously by the methods here described.

A question of great interest would be to obtain an accurate expression of the condition existing between the increased carbon dioxide over-output after work and the lactic acid concentration in the blood. One must presume that areas where the ventilation is lowered, are to be found in certain cases in tuberculous lungs but where the circulation of blood is maintained. If this is correct, there will thus arise »dead areas» where the carbon dioxide tension increases and the oxygen saturation of the blood becomes deficient. This condition might further entail increased ventilation and therefore increased carbon dioxide output, a situation which perhaps would explain the above mentioned (p. 027) lack of agreement within certain clinical groups between the over-output of carbon dioxide after work and the oxygen acceptance during work.

It was our intention to examine this condition experimentally in connection with simultaneous lactic acid analyses in the blood; similarly, we wished experimentally to examine whether a decreased respiration volume leads to an oxygen deficiency during work, with change in the anaerobic work and the consequent gaseous exchange in the lungs. None of these investigations were however carried far enough to enable any definite conclusions to be drawn from the results.

The supply of oxygen to the organism is, besides upon the function of the lung, dependent also upon the blood circulation. For this reason the relations of the latter should be examined in order to determine the respiratory conditions pertaining in tubercular patients. These relations are, however, very complicated and will require a detailed investigation.

Summary.

These investigations were undertaken in order, if possible, to get closer to the question of a simple lung function-test.

A method was used, partly new, which permitted of a protracted and continuous registration of the respiration during rest as well as during and after a brief task. A series of tests were made

with normal individuals (19 men, 21 women) as well as persons suffering from pulmonary tuberculosis (84 men, 75 women).

Through a statistical treatment of the results it was found that patients usually showed a lower oxygen acceptance during work with a following surplus of carbon dioxide output and ventilation, causing certain characteristic changes in the shape of the curves. This is supposed to be an expression of the oxygen supply to the working muscles being less than normal and the anaerobic work correspondingly greater that again will result in a higher concentration of lactic acid in the blood.

An approximate correlation was found between the pulmonary changes and the respiratory results. The method used will, however, not permit any certain conclusions with regard to individual lung function-tests and therefore it does not fit for clinical use.

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Vitamin D Intoxication in a Case of Parathyroprival Tetany.

Report of a Fatal Case with Autopsy Findings.¹

By

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(Submitted for publication February 18, 1948.)

That vitamin D in excessive doses sometimes is apt to act deleteriously upon the system has been revealed both by experiments on animals (Agduhr (1), Bufo (2), Steck, Deutsch, Reed and Struck (3)) and by clinical observations (Putschar (4), Thatcher (5, 6), Bufo, Tumulty and Howard (7), Danowski, Winkler and Peters (8), Freyberg and Bauer (9), Jelke (10), Debré and col-lab. (11)). Hypercalcemia then ensues, and in consequence of this, tissue changes generally involving the walls of blood vessels and adjacent tissues but also the parenchymal elements of internal organs, *e. g.* those of the lungs (11), myocardium and kidneys. The changes are characterized by edema, fibrinous degeneration of connective tissue, necrosis and calcification. Of the internal organs the kidneys seem to be especially vulnerable (2, 3, 4, 5, 6, 7, 8, 10, 12), a circumstance that is probably connected with the increased demands made upon the organ by the excretion of urine rich in calcium. The histological picture does not contradict this assumption, characterized as it is by degeneration and calcium deposits in the tubular epithelium and by tubuli filled with masses of lime. In some cases these calcium deposits may be so considerable that they become grossly visible in the form of white streaks at the border between cortex and marrow (Putschar

¹ The writer expresses his gratitude towards Senior Physician G. Brun, M. D., who made the case available to him.

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(4), Johnsson and Wilton (13) 1 case). A point of practical importance is that the changes may be reversible, if the administration is discontinued in time. Otherwise they will become progressive, so that eventually the patient's life is endangered.

In 1937 Steck, Deutsch, Reed and Struck published a paper on vitamin D poisoning, based upon comprehensive experiments with dogs. A dose of less than 20,000 I. U. per kg body-weight and day did not give rise to conspicuous injuries, not even after months of treatment, while the majority of animals that received larger doses died within a comparatively short time. Microscopic examination and chemical analysis showed that the most pronounced changes were located in the kidneys; the dry substance contained up to 10 times as much calcium as normal kidneys. The authors also give a table of toxic symptoms in a clinical material comprising no fewer than 773 patients who had been treated with vitamin D in doses of 100,000—200,000 I. U. per day. The majority of patients were subjects suffering from allergic diseases, hay fever and asthma (500); some had arthritis, 2 had post-operative tetany. In 63 patients, *i. e.*, in 8 % of the cases, toxic symptoms were noted which in all the cases disappeared again when the treatment was interrupted.

Also in 2 cases of hypervitaminosis D published by the present writer (10) the prognosis was good, although in one of the cases there were severe toxic symptoms including, *inter alia*, an absolute renal insufficiency. In the 2 cases described by Tumulty and Howard (7) in 1942 (ergosterol intoxication in young men with fractures, where treatment with huge doses of vitamin D had resulted in renal injury), reduced renal function was demonstrated even after the lapse of several months; and the same applied to Danowski, Winkler and Peter's 2 cases. In one of the latter there was a certain increase of non-protein nitrogen even $2\frac{1}{2}$ years after termination of the vitamin treatment.

If the injurious agent is allowed to continue exercising its influence after toxic injuries have once been established, these will progress and become so extensive that they will finally be incompatible with the continuation of life. The dose and period of treatment leading to this eventuality are subject to individual variations. The appearance of symptoms of intoxication is favoured if the medication takes place in summer, as a not inconsiderable *natural* D-production occurs in the skin under the influence of the ultra-violet radiation, and also if the agent is taken in milk,

absorption of the fat-soluble vitamin then being more rapid and complete (Lewis, quoted by Tunnally and Howard (7)). Since one of the specific functions of vitamin D consists — as is generally acknowledged — in increasing the calcium resorption (or decreasing its re-excretion via the intestine) the calcium intake with the food may be of some importance. Cf. Jones' investigations (14) with rats, which showed that *small* amounts of vitamin D can produce a pronounced hypercalcaemia if the diet is rich in calcium but poor in phosphorus. The larger the amount of calcium ingested with the food, the more easily are signs of hypervitaminosis provoked (15). Freeman, Rhoads and Yeager (12) studied the calcium metabolism in a boy aged 9 with chronic rheumatoid arthritis under treatment with vitamin D (Eutron) 100,000 I. U. per day. They found vitamin D administration in conjunction with *high* calcium diet, including 1 quart of milk and corresponding to 1.4 g calcium per day, to increase the calcium output both in the urine and stools. With a *low* calcium diet (0.2 g per day), the fecal excretion of calcium decreased, whereas there was a rise in the urinary excretion of calcium.

The first to report a fatal case of vitamin D intoxication was Putschar (4) (1929), and subsequently a further 10 cases have been described, as will be seen from the table. As the danger of an over-dosage of vitamin D in human subjects does not seem to be generally appreciated, the report of the following case should be considered justified.

Mrs. A. S., born 11/4/1891, treated in the Medical Department of the Central Hospital in Gävle 6/4—6/5 1945 under the diagnosis *Intoxicatione vitamini D*. Nephrosclerosis. Anemia.

From the case history: In 1926 the patient was operated upon for struma, probably atoxic, which had caused hoarseness. About 1928 she was in other respects quiet fit and doing full-time work until 1938, when she began to feel faint and nervy and to suffer from blurred vision (both eyes). In 1939 she was afflicted with tonic cramp in the hands, sometimes also in her feet and legs. The cramp was painful and lasted for 5—10 minutes at a time. In the same year she was operated upon for *Cataracta tetanica* in the left, and 1940 also in the right eye. The blood calcium, which on the first occasion was 8 mg%, had in 1940 dropped to 6.3 mg%. A. T. 10 was now administered in a dose of 30 drops every other day, during which treatment the blood calcium rapidly rose to normal and the above mentioned symptoms disappeared. She continued after

Table
Reported Cases of Vitamin D

Case	Age	Sex	Preparation	Dose/Day
1. Putsehar (4) 1929	5½ months	M	«Altvigantol»	6 drops
2. Thatcher (5) 1931	18 months	M	Viosterol (Ergosterol emulsion)	4 teaspoons
3. Thatcher (6) ¹ 1936	11 ½ months	M	Cod liver oil (200 IU/g)	400 to 1200 IU
4. Kerr, quoted by Steck and collab. (3) 1937	74 years	M	Viosterol in con- centrated solu- tion	2,300,000 IU
5—6. Ross and Williams (16) 1939	8—14 months	M	«Ostogen» (Irrad- iated ergosterol 60,000 IU/g)	20,000 to 40,000 IU
7. Wolf (17) 1943	4 months	M	«Ertron» (electric- ally activated er- gosterol)	300,000 IU
8. Freyberg and Bauer (9) 1945	32 years	F	Vitamin D «Plasta Seal»	100,000 to 500,000 IU
9. Jääskeläinen (18) 1946	8 months	M	D ₂	470,000 IU
10. Jelke 1948	54 years	F	«Ultranol fortior» 250,000 IU D ₂ /ml	250,000 IU

this to take A. T. 10 for 3 years, from 1942 20 drops a day. When for a trial period she reduced the dose to 10 drops daily slight cramps once more appeared. At the yearly follow-up examinations at a university

¹ In view of the low daily dose of vitamin D used in this case, the question arises of whether there has possibly been a *primary* renal lesion enhancing the sensitiveness to this vitamin.

1.

Intoxication with Lethal Course.

Duration	Season	Major Pathology
96 days		Calcification in the tubular epithelium, basement membrane and interstitial tissue of the kidneys.
5 months	Summer	Deposition of calcium chiefly in the lumina of the collecting tubules of the kidneys. However there were also pus cells, indicating infection of the urinary tract.
4 months	Summer	Calcification in the lumina and in the cells of the collecting tubules of the kidneys. Parenchymatous degeneration of the epithelium of the tubules.
18 days		
Several months		Twins. The cases are not pure, since both were suffering from bronchopneumonia. In the one case asphyxia through aspiration was the immediate cause of death. Post-mortem showed here extensive metastatic calcification in the kidneys, stomach, lungs, and in the inner layer of the media of the arteries. In the other case, where post-mortem was refused, the X-ray examination of the long bones had shown increased density at the zone of the provisional calcification.
14 days	Spring	Numerous calcium deposits in the kidneys, especially in the tubuli.
1 year	Summer and winter	Calcification in the kidneys, heart and arterial vascular system.
7 days	Autumn	Thrombosis in the one renal vein with necrosis of corresponding kidney. Nephrotic changes in the other. In both, calcium cylinders in the tubules. Numerous calcium deposits in the walls of the pulmonary alveoli.
1 year 3 months	Summer and winter	Subcutaneous nodules with necrosis of the arterial walls and adjacent connective tissue. Coronary sclerosis, ample calcification of the aorta. Nephrosclerosis, peculiar calcification of the fine interstitial arterioli of the kidneys.

clinic the patient's condition was satisfactory: the blood calcium, and also the urine, proved normal on every occasion. The basal metabolism was estimated in 1941, viz. + 5 %, and in 1943, viz. — 5 %.

In September, 1943 the A. T. 10 was replaced for financial reasons with the cheaper Ultranol fortior, *i. e.* crystalline vitamin D₂ dissolved in peanut oil, of which the patient subsequently took 1 cm³ = 250,000

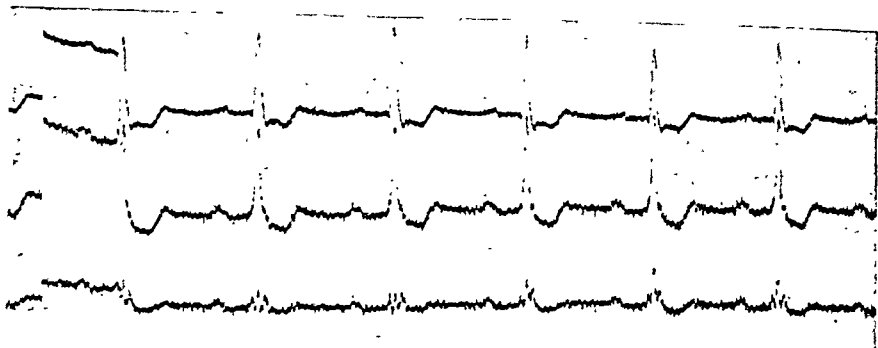


Fig. 1.

I. U. every day right up to December 1944. After 5 months' treatment there appeared an eruption on the fore-arms, which left behind firm callous nodes. After this the patient's condition began to become successively worse, with general lassitude, dyspnea on exertion, swollen legs, constant thirst and nycturia. Further, itching over entire body. In spite of good appetite she lost almost 20 kg. In October 1944 she consulted a physician, who found increased blood pressure (275) and albuminuria. Two months later she stopped taking Ultranol fort., as she thought she was not able to tolerate the drug. Continued with A. T. 10 without any improvement.

From the findings on admission, 6/4 1945. General condition unimpaired. Skin somewhat pale, moderate edema in legs. On the extensor aspects of the fore-arms a number of hard, callous nodes of pea to bean size in the subcutis. A couple of similar nodes on the flexor aspect of the left fore-arm, one on the palm of the left hand. Right lobe of thyroid slightly enlarged and firm. Lungs, N. A. D. Heart: 3 + 11 cm. Systolic murmur strongest over the apex. Second aortic sound, accented. Rhythmical. Pulse 90. Blood pressure 185/75. Abdomen, normal. Electrocardiogram: Rhythm, regular; heart rate, 85. P—Q 0.20 sec. QRS 0.09 sec. Ventricular complex, 0.28 sec. (normally 0.28—0.36 sec. according to Ljung). S—T considerably depressed in all leads. T pos. Diagnosis: myocardial injury.

Laboratory findings. Urine: acid. Spec. w. 1.012. Alb., traces. Sacch., negative. Rather abundant granular cylinders in sediment. Blood: Hb 55 % (Sahli). Reds 3,160,000; Whites 4,000. S. R. 70; rose successively to 110. N.P.N. 135 mg%. Blood calcium 12 mg%, blood phosphorus 5 mg%. Subsequently there was constant hypercalcemia of between 14.5 and 17 mg%; on 27/4 the maximum rate of 20 mg% was noted. Citric acid, 48/ml, phosphatase, 6 units.

X-ray findings. No calcification visible in the scout skiagram of kidneys and urinary tract. The heart shows some hypertrophia of the left ventricle with elongation of the aorta, but the volume is normal.

Biopsy of node excised from left fore-arm. The specimen consists of fatty connective tissue, showing marked changes partly in the form

of scarred areas with peculiar fibrinous degeneration of the collagenic tissue, and partly in the form of necrosis, which involves, *inter alia*, also the wall of a fairly large arterial branch (Gellerstedt).

Course of disease. The patient was treated with digitalis and ammonium chloride, and received in addition Ultranol fortior 1 ml \times 1 the first 2 days. The first 10 days the temperature was subfebrile to 37.9° C., subsequently being normal. The blood pressure dropped to 150/70. The amounts of urine, which at first were rather large (up to 2,500), after 10 days diminished successively until complete anuria ensued. Specific weight between 1.006 and 1.015. There were in general traces of albumin, while the sediment was normal, until eventually white blood corpuscles and bacteria appeared. N.P.N., which on 12/4 was 100 mg%, then rose continually to 277 mg% on 3/5. Hand in hand with this the general condition grew worse; the patient became increasingly apathetic, finally comatose and died on 6/5.

Autopsy. Lungs emphysematous. Hypostasis and edema in declivous parts. A few small purulent clots protrude from the incision. *Heart.* The mitral valves markedly thickened, annulus fibrosus harder than normal. The left ventricle hypertrophic, the walls measure 25 mm. No myocardial cicatrices. The coronary vessels wide to begin with but peripherally sclerosed, and the aorta shows here and there abundant lime salt incrustation. The liver somewhat enlarged, firm with blurred texture. The *kidneys* weigh 100 g each. The capsule loosens with some difficulty, whereupon a smooth renal surface with pronounced vascular network is exposed. The vessels are dilated. The incision shows a pale parenchyma with very thin cortex.

Patho-anatomical Diagnosis: Lungs: hemorrhagic broncho-pneumonic foci. Heart: slight acute disseminated myocarditis, moderate coronary sclerosis. Liver: slight acute disseminated hepatitis. *Kidneys:* nephro-sclerosis with certain nephritic features and peculiar calcification of the fine interstitial arteries. Main cause of death: renal affection.

Comment. A 54-year-old woman who at the age of 35 was operated upon for an atoxic struma had for the next 12 years been on the whole fit and able to work, except for some ache and swelling of the legs. She was then afflicted with tonic cramp in the extremities and cataract in both eyes, which was operated. As hypocalcemia was observed 14 years after the strumectomy, A. T. 10 was administered with satisfactory result. The blood calcium rapidly rose to normal, and the cramps and aches subsided. Three years later the A. T. 10 was replaced with vitamin D₂, of which she took 250,000 I. U. daily for one year and three months. After 5 months' treatment, there developed a number of hard nodes on the fore-arms, and after this her condition began to become progressively worse, with general lassitude, dyspnea on exertion, swollen legs, constant thirst and emaciation.

The objective examination showed hypercalcemia, myocardial injury, hypertonia and symptoms of absolute renal insufficiency. Biopsy of one of the above-mentioned nodes on the fore-arms showed fibrinous degeneration and necrosis in the subcutaneous connective tissue as well as in the arterial walls. A certain compensatory polyuria was followed in a short time by oliguria—anauria, and increasing azotemia culminated in death. The post-mortem examination, including microscopy, revealed above all serious changes in the kidneys: sclerosis and extensive calcification in the fine interstitial arteries.

Discussion.

It is evident that subsequent to the strumectomy a parathyroidal tetany developed, despite the fact that on account of the masked symptoms the diagnosis remained obscure until 12 years later, when there already was a cataract. A further 2 years passed before the patient had the benefit of appropriate treatment with A. T. 10. If this had been started in time it would probably have been possible to prevent the ocular complication altogether.

A. T. 10, dihydrotachysterol, which is obtained as a fraction by irradiating ergosterol, was introduced, as is known, by Holtz (19) about 10 years ago as a remedy for cases of tetany, whether post-operative or so-called idiopathic. Thanks to this treatment, numerous patients have recovered health and working capacity, provided it has been carried out *lege artis* with regular controls, particularly of the blood calcium, the only sure criterion for the dosage. That this precaution should be observed no less strictly as regards treatment with vitamin D₂ in huge doses, as has been suggested for tetany especially by American authors (Severinghaus (20), see also Waldenström (21)), is illustrated by the case reported. When the patient had had this treatment for 5 months, well-defined tissue changes appeared, which may reasonably be interpreted as being due to hypervitaminosis D, to which are added adynamia and emaciation, which the writers on this subject are unanimous in describing as the early symptoms of experimental D-intoxication. The clinical picture is completed by signs of relative heart insufficiency and also by polydipsia and polyuria, symptoms that have been observed and described by several authors in connection with D-hypervitaminosis (3, 7, 10, 11). This diagnosis is also confirmed by the objective examination through the demonstration of a marked and stable hypercalcemia.

Further, the entire picture presented by this case, as regards both its clinical and patho-anatomical aspects, will be easily understood on this account. The biopsy performed on one of the nodes in the fore-arms disclosed tissue and vascular changes of the D-hypervitaminosis type, which on the whole tallied well with those observed by Johnsson and Wilton (13) in 4 infants suffering from congenital malformations and treated with vitamin D in massive doses. These lesions were characterized by edema in the media with disintegration of the elastic elements and more or less extensive necrosis, in some cases with lime salt deposits. The absence of calcification in our case should undoubtedly be considered a difference rather in degree than in nature.

Under the conditions obtaining, it is highly probable that the vitamin D intoxication may also have played a part in the origination of the advanced sclerosis involving central parts of the vascular system, viz. the aorta and coronary arteries, although in a subject of the patient's age atherosclerosis proper cannot be ruled out definitely. On the other hand, Gerlach (22) has described atherosclerosis of the aorta and coronary arteries in a girl aged 9; at the age of 2, this patient had been given 18,000 I. U. vitamin D₂ daily for one month, and subsequently 12,000 I. U. daily for five months, *i. e.* doses that, calculated per kg body-weight, amounted to merely $\frac{1}{10}$ — $\frac{1}{3}$ of those taken by our patient. In addition, similar vascular lesions have been produced experimentally in animals.

The relative cardiac insufficiency observed in our case was due to myocardial injury, which was probably secondary to the sclerosis of the coronary vessels (*cf.* the electrocardiogram, with considerable depression of the S—T segments). Whereas formerly myocardial lesions had not quite infrequently occurred following treatment with less pure preparations of vitamin D (Agduhr and Stenström (23), Herlitz, Jundell, and Wahlgren (24), Wernstedt (25)), the highly refined preparations available at the present time do not seem to entail any actual danger in this respect. EKG abnormalities with prolongation of the conduction period have however been observed by G. Tumulty and Howard (7), as well as by Olsen (26), in one case each. The duration of the ventricular complex (Q—T), in the case in hand approaching the lower limit of normal, in some few cases of hypervitaminosis D was noted to be decidedly shortened (Jelke (10), Olsen (26) (2 cases)), probably due more directly to the hypercalcemia. As a homologous

phenomenon might here be quoted the EKG changes associated with hyperparathyroidism (Korth and Hecht (27)), the most conspicuous biochemical feature of which is just hypercalcemia.

Actually the syndrome as well as the course of the disease was dominated by renal symptoms, also in conformity with the majority of published cases of D-hypervitaminosis. The absolute renal insufficiency progressed and within a short time resulted in death from uremia. That the two D₂ doses administered to the patient during the first days played a rôle in this development cannot be entirely ruled out. At the post-mortem examination nephrosclerosis was found, as well as extensive peculiar calcified foci in the fine interstitial arteries. It is perhaps not too bold to assume that these vessels had first been the site of processes similar to those observed in the arteries of the subcutaneous connective tissue, or in other words that we are confronted with a toxic vitamin D effect also here, despite the fact that no typical lime salt metastasis was demonstrated. The possibility that the nephrosclerosis had arisen *per se*, while vitamin D₂ was being given, but independently of this, cannot be entirely refuted; but in this case the treatment adopted must nevertheless be held responsible for the development of the pathological condition. (See below.)

That a D-intoxication had occurred and played an essential rôle for the actual issue in this case must thus be considered to have been established. *How* did it then arise? As regards first of all the D-dose administered, 250,000 I. U. per day, corresponding to about 4,000 I. U. per kg body-weight and day, this is not exceptionally high, but is considerably below the maximum dosage, 20,000 I. U. per kg body-weight, that according to Wolf (17) may be safely administered to adults for an indefinite period. The literature is, however, not without instances of hypervitaminosis developing after much smaller doses. In Danowski, Winkler and Peters's (8) case 1, a 47-year-old woman who on account of chronic polyarthritis took vitamin D 150,000—200,000 I. U. per day, hypercalcemia, calcification of soft tissues and renal injury developed after 6 years' medication. And in the above-mentioned clinical series of Steck and his associates, where symptoms of intoxication appeared in 8 % of the cases, the dosage adopted was not higher. According to Parks, a daily dose exceeding 40,000 I. U. (= 1 mg) is to be regarded as potentially dangerous (15).

One should, however, take into consideration the possibility

that some special factors may have contributed to the appearance of the symptoms of intoxication in our case. As regards first of all the 3 years' A. T. 10 treatment, which preceded the D-treatment, it is not entirely out of question that it played some rôle. Both the substances are derivatives of ergosterin with a similar action upon the system, as has been shown by, *inter alia*, Sprague, Haines and Power (28) in a case of «pseudo-hyperparathyroidism» Albright. An increase in the blood calcium with diminution of the calcium excretion with the stools was obtained with both the preparations, resulting in a more positive balance for both calcium and phosphorus. No symptoms of intoxication, it is true, appeared during the A. T. 10 treatment, but it is not entirely inconceivable that it may have increased the sensitiveness to the immediately following vitamin D treatment. By way of comparison, mention may be made of Olsen's 2 cases of parathyroidectomy, which for 6—7 years had been treated with A. T. 10 when they were started on pure vitamin D₂ (Ultranol fortior). In both these cases there appeared after the lapse of 6—10 months, in addition to general symptoms of intoxication with hypercalcaemia etc., well-defined calcified foci in the soft tissues (para-articularly), despite the fact that in one of the cases the dosage used had not been higher than 60,000 I. U. per day.

As the treatment in our case was continued during the summer, a seasonal intensification of the D-effect should be taken into account. And such an intensification was achieved also by the patient's generally taking the drug in milk.

Vitamin A deficiency, like vitamin B deficiency (Jung (29)), is supposed to increase the sensitiveness to vitamin D; but in our case there was no evidence of either the one or the other of these two conditions. A slight hypothyroidism, on the other hand, which according to Steck and his associates is believed to have a similar effect, cannot be entirely ruled out. The patient, who in 1926 underwent strumectomy, had 1943 a basal metabolism rate of —5%. For example mention may be made of one of Olsen's cases of D-hypervitaminosis that had earlier been treated for myxoedema strumipriva.

An important factor for the estimation of the tolerance to vitamin D is the functional status of the kidneys, for it is upon this to a great extent that the *duration* of a hypercalcaemia eventually provoked is dependent, and therewith the consequences of the latter for the system as a whole. In a child with severe renal

injury insufficient D intake does, it is true, easily lead to so-called renal rachitis; but on the other hand, larger doses (which for healthy persons entail no risk whatever) entail the risk of metastatic calcification of blood vessels and organs (17). If in our case any renal injury *sui generis* arose imperceptibly in the course of the treatment and independently of this, which no doubt is a mere hypothesis, this would give rise to enhanced sensitiveness to vitamin D with possible toxic effect from even »moderate» doses as a consequence. That in such a case continued D-treatment must actively contribute to irreparable changes and possibly to a fatal issue is evident.

As I have pointed out in a previous paper, every renal affection must be regarded as a definite contra-indication against D-treatment in the form in question, as had also been stressed by Steck and his associates.¹

Conclusion.

Since, as has been shown on repeated occasions, vitamin D in huge doses is not without danger to the system, a prolonged treatment with this can as little be carried out without continual examination as in the case of A. T. 10. In this connection control tests should be carried out not only in respect to the blood calcium but also to the body-weight, blood pressure and urine. The case here under discussion — like the majority of previously reported cases of D-intoxication — teaches us that the kidneys especially are easily injured, so that due attention always should be paid to the state of these organs. As incipient renal injury generally manifests itself first of all through reduced capacity of concentration, the determination of the specific weight of morning urine or after concentration tests is of course an important part of the examination. At the first sign of renal injury the treatment must be discontinued at once, in order as far as possible to obviate the development of irreparable changes. In cases with high calcium diet, *e. g.* with ample ingestion of milk, the calcium intake should be restrained.

Summary.

A case of post-operative parathyroprival tetany leads after the lapse of 12—14 years to bilateral cataract, which is ope-

¹ In the opinion of Freeman and his associates, »such a contra-indication does not apply unless there is »evidence of impaired renal function».

rated. In connection with this the true nature of the patient's symptoms is revealed, and appropriate treatment, viz. A. T. 10, is instituted. Three years later this drug is replaced with a synthetic D₂-preparation (Ultranol fortior), of which during a period of 1 year and 3 months the patient takes 250,000 I. U. per day. After 5 months' treatment hard nodes situated in the subcutaneous tissue appear on the arms. These prove to correspond to necrosis in the arterial walls and adjacent collagenic tissue. Subsequently there is increasing adynamia, dyspnea on exertion, swelling of the legs, emaciation, polydipsia and nycturia. The objective examination shows signs of myocardial injury, hypercalcemia and azotemia. The non-protein nitrogen continues to rise steadily, at the same time as the amounts of urine diminish to the point of complete anuria, and the patient dies of renal insufficiency (uremia). The principal post-mortem findings are renal injury with nephrosclerosis and extensive calcification of the fine interstitial arteries.

The case is interpreted as one of vitamin D intoxication with renal and vascular injuries. Had the nephrosclerosis possibly commenced independently of the vitamin D administration, this will nevertheless be mainly responsible for the continued course of the disease, as in such a case the sensitiveness to vitamin D is enhanced.

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The Relation Between the Dosage of Desiccated Thyroid and its Effect on the Oxygen Consumption in Healthy Individuals.

By

HANS DAHLSTRÖM and TORGNY SJÖSTRAND.

(Submitted for publication December 23, 1947.)

The thyroid preparations used in therapy are standardized either chemically, by determination of the iodine content, or biologically, by animal tests. Sometimes, only the amount of desiccated gland corresponding to the preparation is indicated. The chemical standardization does not supply a direct indication of the amount of effective hormone, as the iodine content is probably influenced by the amount of other iodine containing substances than »the thyroid hormone» (Dressler and Hölling, 1940). Furthermore, it has not been proved that the relations between dosage and effect obtained in animal experiments can be applied directly to the human being.

It has also been a clinical experience that occasionally the expected raise of the basal metabolic rate is not found when employing thyroid preparations for the purpose of reducing weight. This is sometimes blamed on the inferior effect of a certain preparation, but it can also be explained by supposing that there is no direct relation between dosage and effect of the thyroid preparations.

The increase of oxygen consumption has earlier been accepted as a measurement of the increase in the metabolic rate, produced by the thyroid preparations. We have therefore considered it to be of interest to determine the relation between the dosage and

the effect on oxygen consumption of commercial preparations of desiccated thyroid in normal individuals under standardized conditions.

Method.

The determinations of the oxygen consumption were all performed in volunteers, who generally came to the hospital by tram, or if not, travelling slowly on a bicycle. The determinations were performed after a 30 minutes rest, and the test itself lasted 10 minutes. Only one determination was performed at each day.

In some test series, the oxygen consumption was determined by measurement and analysis of the expired air, and in others according to the method of Krogh. In the former series the expired air was collected in a Douglas bag and the determination of O_2 and CO_2 was carried out according to Haldane's method. This procedure is less open to errors than is the method of Krogh, when a trained personnel is available. The »bag method» is less discomforting to the test person, especially in those cases where there is a considerable increase in the oxygen consumption, and is therefore preferable in these cases, as also when the mean respiratory level varies during the experiment. The Krogh method was used when there was no special reason to employ the bag method.

11 men and 1 woman have volunteered as test persons. Introductory determinations were also performed in an additional group comprising 7 men and 1 woman. These subjects, however, proved unsuitable, as their oxygen consumption varied a great deal from day to day. Sex, age, height, weight, B. M. R. (expressed as % of the normal values, calculated according to Harris and Benedict), and oxygen consumption in the different subjects are represented in table 1.

As most of the subjects showed a reduced oxygen consumption at each successive determination for some time, due to their getting acquainted with the arrangement of the experiment, it was necessary to eliminate this source of error. This »training» may require a shorter or longer time, in different individuals, as has been investigated by Vogelius (1945). Therefore the experiments were conducted thus, that repeated determinations were made on different days, until approximately the same values of oxygen consumption were obtained at several consecutive

Table 1.

Subject	Age	Height in cms.	Weight in kgs.			B. M. R. ¹		O ₂ consump- tion in mls/ minute after »training»	Days required to reach equilibrium
			Begin- ning	Highest	Lowest	First de- termina- tion	After »train- ing»		
F. L. ...	25	173	65.3	67.1	65.3	96 %	102 %	240	8
L. S. ...	24	177	68.5	69.0	67.3	107 %	101 %	248	9
K. H. ...	22	180	68.3	69.9	67.8	101 %	100 %	249	6
F. N. ...	24	181	71.2	71.2	70.3	103 %	100 %	253	4
T. B. ...	27	170	64.1	64.8	64.0	125 %	103 %	236	12
N. S. ...	23	177	67.6	67.6	65.2	109 %	101 %	245	10
C. L. ...	25	179	99.5	99.5	97.8	88 %	100 %	304	5
T. H. ...	22	179	66.0	67.0	66.0	83 %	89 %	218	5
N. Q. ...	36	180	78.0	79.0	78.0	93 %	95 %	241	5
B. S. ...	23	172	78.4	78.4	76.3	93 %	88 %	231	8
H. D. ...	25	174	76.5	77.6	76.5	87 %	91 %	233	6
I. N. ...	22	161	56.0	56.7	55.6	96 %	95 %	186	16

determinations. The administration of thyroid preparations was generally not instituted until four oxygen consumption values, obtained at consecutive determinations, proved to remain at approximately the same level, thus not tending towards increase or decrease. Data concerning the effect of the accommodation and the time required to reach a stable level in each case, are found in table 1.

Once a stable level was reached, thyroid was administered, employing commercially available tablets and a certain daily dose was maintained until a new level of the oxygen consumption was reached. Then the administration was discontinued, and in some series the return to the level prior to the medication was followed by consecutive determinations of the oxygen consumption. In most series, however, there was an interval of 1—3 weeks before any new determinations were made. When in these cases a new level was reached as proved by at least four consecutive determinations, a second period of administration was started, and the experiment thus repeated. In some experiments the dosage in the second series was greater than in the first one, whereas in others it was smaller.

When judging the effect of a certain thyroid dosage, we have generally considered the mean value of 3 or more consecutive determinations, performed at different days, and showing no

¹ »First determination» represents one single value, »after training» a mean value of 4 determinations, consequently, these values cannot be directly compared.

decided tendency towards increase or decrease. When employing larger dosage, however, we have not been able to follow this rule, as it proved difficult to induce the subjects to continue the medication long enough, when the toxic symptoms became more accentuated. In these cases we have determined the mean value of 3—4 determinations, obtained after a time corresponding to the time when a new level was reached in the cases where a lower dosage was employed.

We have chiefly used the thyroid preparation »Thyreototal Astra». »Thyroid gland» (Burroughs, Welcome & Co.) and »Thyroid gland» (Park, Davies & Co.) have been only administered in some comparing experiments.

Results.

By determination of the effect of a certain dosage of thyroid, it proved that the values of oxygen consumption reached an equilibrium level after approximately 9 days, when employing low and moderate dosages. This is shown in table 2, which comprises the values of oxygen consumption obtained at different intervals from the beginning of the administration. In parts this corresponds to the observations made in rats by Mörch (1929). In addition, Mörch found that it takes a longer time to reach an equilibrium with a low dosage than with a higher one. We have not been able to confirm this fact in the human being. As was mentioned above, we were not able to continue the administration of high doses until a satisfactory equilibrium was reached, as the subjects were bothered by the toxic effect. From our results, however, it does not seem probable that this could be demonstrated in man.

In the series employed in the investigation of the relation between the amount of administered thyroid and the oxygen consumption, thyroid was administered for a minimum time of 2 weeks.

We regard the level of oxygen consumption attained on the 9th—14th day as the effect of the preparation at the dosage in question, even though the oxygen consumption tended towards an increase after this time in certain experiments, as is evident from table 2. This approximation, however, which has been done for practical reasons, we consider justified.

Table 2.

O₂-consumption Different Days after Initiation of the Thyroid Medication.

Subj.	Preparation	Dosage		O ₂ -consumption in mls/min.				
		mgsI/day	gms thyr. sicc/day	Prior to medication	5th—10th	10th—15th	15th—20th	25th—35 th day
F. L.	Astra...	0.12		240	230		228	
	» ...	0.36		241	248	248		
	» ...	1.08		238		274, 284, 286, 299		
L. S.	» ...	0.12		248	246	257		
K. H.	» ...	0.36		249	230	245	240	
	» ...	1.08		249	267	281		
F. N.	» ...	0.12		253	254	240		
	» ...	0.36		248		272	274	
T. B.	» ...	0.36		236		245	245	265
	Burroughs, Welcome & Co.....		0.15	—		258	260	
N. S.	Astra...	0.36		245			262	292
	Burroughs, Welcome & Co.....		0.15	—		257	251	
C. L.	Astra...	0.36		304		340	342	
N. Q.	» ...	0.36		241	239	251	250	
	» ...	0.72		243	254	260		
B. S.	» ...	0.18		231		246	240	
	» ...	0.36		233		237	241	
	» ...	0.72		236	265	263	300	
I. N.	» ...	0.12		186	182	183		

In fig. 1, the relation between dosage and effect in the different subjects has been summarized, and furthermore an approximate mean value curve has been drawn. Only the series in which »Thyreototal Astra» was used, are included here. It is evident from the diagram, that there is no simple relation between the dosage of the preparation and the change in oxygen consumption. Thus, at low and moderate dosage there is a decrease in the oxygen consumption, or now and then, an unaltered consumption.

This implies that thyroid can have both an increasing and a decreasing effect on the oxygen consumption. The latter effect occurs with low and moderate dosage. It deserves to be mentioned that in our experiments we have excluded the possibility, that the decreasing effect of the low and moderate doses should be

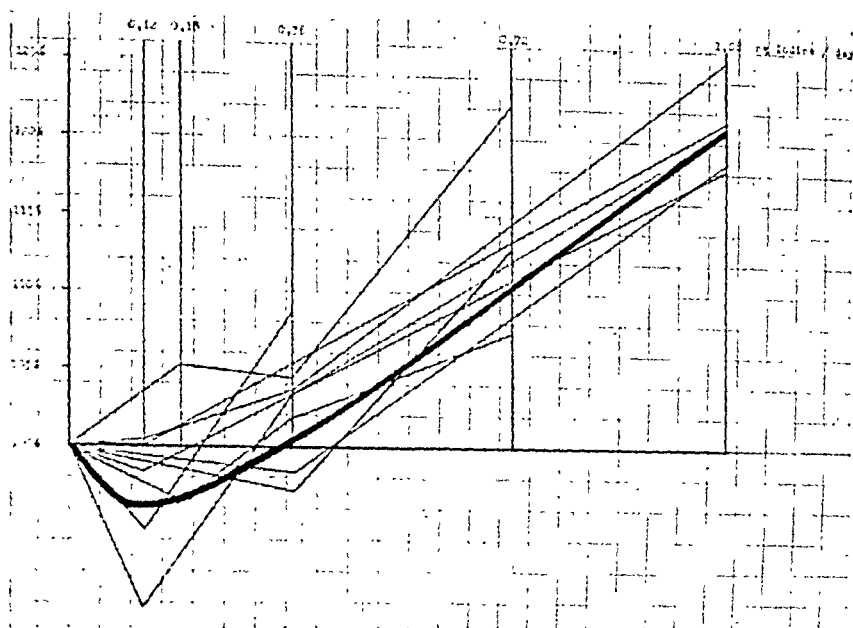


Fig. 1. The relation between the dosage of thyroid preparation and the change in oxygen consumption (expressed in %). Ordinate: Oxygen consumption. Abscissa: Thyroid in mg l/day. The thick drawn line is an appr. mean value curve.

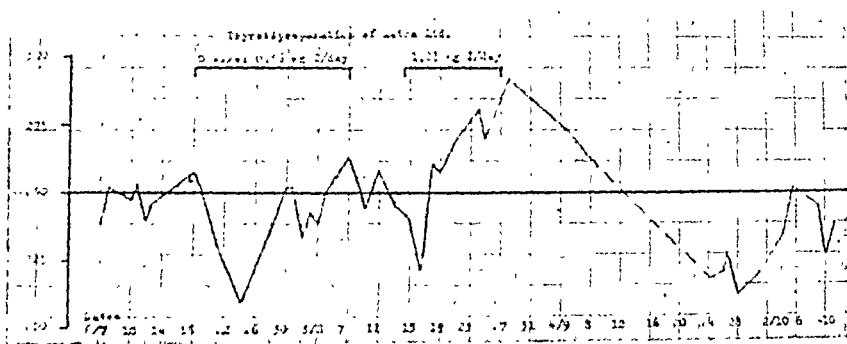


Fig. 2. See the text. Subject K. H. Ordinate: Oxygen consumption expressed in ml. O_2 /min. Abscissa: Time in days.

caused by habituation. As has been mentioned above, we did not start the administration until an equilibrium level was reached, and we have furthermore controlled that the oxygen consumption did return to the original level after discontinuing the medication.

It appears that this double and antagonistic effect is also encountered at a more detailed analysis of the different values

Table 3.

Comparison of the Effect on O₂-consumption of Different Commercial Thyroid Preparations.

Subj.	Preparation	Dosage		Oxygen consumption in mls/min.		
		mgsI/day	thyr. sicc/day	Prior to medication	9th—14th day	Increase in %
K. H.	Astra.....	0.36		249	245	— 2 %
	»	1.08		249	281	+ 13 %
	Park, Davies & Co., Burroughs, Welcome & Co.....	1.08	0.36	237	281	+ 19 %
	— » —		0.15	228	214	— 6 %
			0.30	228	280	+ 23 %
T. B.	Astra.....	0.36		236	245	+ 4 %
	Park, Davies & Co., Burroughs, Welcome & Co.....	0.36	0.12	—	248	+ 5 %
			0.15	—	258	+ 9 %
N. S.	Astra.....	0.36		245	262	+ 7 %
	Park, Davies & Co., Burroughs, Welcome & Co.....	0.36	0.12	—	272	+ 11 %
			0.15	—	251	+ 2 %

of oxygen consumption in the individual series. Fig 2 gives an example of the oxygen consumption before, during, and after the administration of 2 different dosages in one of the subjects (K. H.). As can be seen from the diagram, the oxygen consumption diminishes on the 2nd—5th day, and the decrease is greater and more prolonged at a low dosage than at a higher one. This has been the case in 12 of 16 experiments on 12 test persons where we have followed the oxygen consumption continually, even though it has not been quite so evident in all these cases as in this one.

It is also evident from fig. 2, that the oxygen consumption sinks below the initial level after discontinuing a relatively large dosage of thyroid, the initial level not being reached until after 4—5 weeks. This fact was observed in 2 of 3 further experiments series, where the oxygen consumption was followed successively after discontinuing the administration. In the 3rd series (subject B. S.), this decrease was not observed. In this connection, how-

¹ 15th—20th day.

ever, the low initial level in this case is worth mentioning, B. M. R. 88 %.

In three of the subjects, the effects of the different makes of thyroid preparations can be compared (table 3). Thus it is found, that the same doses produces approximately the same effect, and the decreasing effect at low and moderate dosages has been observed both when using *Astra's* and *Burroughs, Welcome & Co's* preparations. The experiments with the preparations manufactured by *Park, Davies & Co.* are too few, to allow any conclusion as to whether there is any real difference between this preparation and the others as regards the decreasing effect. However, a decrease under the initial equilibrium level, after discontinuing a medication of a certain duration has been observed with all three preparations.

Discussion.

The experiments have shown that it may require a comparatively long time before the full effect of a certain dosage of thyroid is obtained, and that low and moderate doses may produce a decrease in the oxygen consumption. This is also the case with larger doses, soon after initiating and also after discontinuing the medication. A decreasing effect on the B. M. R., produced by moderate doses of thyroid in cases of thyrotoxicosis has been reported by Rienhoff (1941). Means (1942) has also suggested the use of thyroid in the treatment of thyreotoxicosis, and mentions that the B. M. R. is lowered when the administration of thyroid in »non-myxedematous hypometabolism» is discontinued.

The explanation of these observations might be, that thyroid preparations have an inhibiting effect upon the thyroid function, either directly on the thyroid gland or indirectly via the pituitary gland. Certain experiments in rabbits (Mahaux, 1938), demonstrating a potentiation of the thyroxine effect by thyreotropic hormone, rather support the latter theory. Lambie (1939) has also published a discussion on this problem.

With a low dosage, and during the beginning of the administration of a thyroid preparation, and also sometimes after discontinuing the medication, the decreasing effect is probably dominating. This might also provide an explanation of the fact, that in-

creased oxygen consumption, caused by the administration of thyroid, can only be demonstrated several days after initiating medication, even though it must be supposed that the effect of the hormone produced in the thyroid gland under physiological conditions has a much more rapid effect. It also provides an explanation to the fact, well known among clinicians, that in order to reach a certain effect it suffices to administer a considerably smaller dosage of thyroid to patients with hypothyreosis than to patients with a normal B. M. R. In this connection it may also be mentioned that it has been a clinical experience, that small thyroid doses may produce a weight increase in emaciated patients.

As we have not used pure hormone preparations in our experiments we cannot say whether the decreasing effect on the oxygen consumption was produced by the hormone itself, or by hormone split products present in the preparations. It would most probably prove of great interest to analyze this question further.

Hitherto we have not found any certain differences between the different preparations available in the open market, as to their decreasing effect on the oxygen consumption, even if such a difference is suggested in our material, which, however, is too small to allow any definite conclusions in this respect.

Neither has this comparison shown any great differences as to the increasing effect, insofar as differently standardized preparations can be compared at all. Here the important question arises as to whether it would not be desirable to standardize these preparations by oxygen consumption determinations in man, thus allowing a more direct comparison between different preparations. It is naturally impossible to make any exact comparison between two different preparations, if one is stated to contain a certain amount of desiccated gland, and the other a certain amount of iodine.

It also seems to us that the above mentioned observations may have a certain practical interest in clarifying how an adequate medication with thyroid should be planned, in order to induce a loss of weight. Thus one should start by giving relatively large doses, and then diminish the dose until a certain increase in the B. M. R., as proved by control, has been reached. This latter increase should be adjusted so, that the medication can be continued over a relatively long time.

Summary.

1. An investigation has been made concerning the effect of thyroid administration on the oxygen consumption in healthy individuals under standardized conditions.

2. With a daily administration of thyroid in low or moderate doses, corresponding to 0.12–0.72 mg I/day, it was possible to change the oxygen consumption in the majority of the subjects, thus reaching a new equilibrium level with an appr. constant oxygen consumption.

3. The administration of large doses 1.08 mg I/day generally had to be stopped, before an equilibrium level was reached because of the subjective complaints on the part of the test persons.

4. Thyroid in small doses generally showed a decreasing effect on the oxygen consumption, as was also in some cases the fact with moderate doses, whereas in a few cases the oxygen consumption remained unaltered.

5. A transitory decreasing effect on the oxygen consumption was observed 2–5 days after the administration of larger doses of thyroid.

6. 2–4 weeks after discontinuing a comparatively large dose of thyroid, that had been maintained for a couple of weeks, the oxygen consumption proved to be less than prior to the medication, subsequently returning to the initial level.

7. The relation between dosage and effect on oxygen consumption has been examined more in detail in 9 individuals. The results are shown in a diagram (fig. 1).

8. A comparison was made between three different commercial thyroid preparations, which did not show any certain differences as to their effect on oxygen consumption. The material was too small, however, to allow any definite conclusions in this respect. It is, however, very difficult to make any direct comparisons between different makes of thyroid preparations, as they are standardized according to different methods. A uniform standardization, based on the effect on the oxygen consumption in man, is suggested.

The costs of this investigation have been granted by Astra Ltd, Södertälje, Sweden.

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Le traitement intraveineux par le fer.

Par

G. HEMMELER,

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1. Les indications de la thérapeutique martiale par voie intraveineuse.

Les états d'hyposidérose réagissent en général favorablement à un traitement à base de fer administré per os. Pourtant, il arrive que l'on assiste à des échecs lorsque, à la suite d'un trouble de la résorption, le médicament n'est pas assimilé, ou quand il est mal toléré et provoque des troubles digestifs tels que nausées, lourdeurs d'estomac, inappétence, diarrhées ou constipation. Ces troubles se manifestent surtout lorsque le fer est mal résorbé ou quand il est pris à de trop fortes doses. Ils s'expliquent aisément: les sels ferreux que l'on prescrit habituellement n'ont pas de pouvoir corrosif sur la muqueuse gastro-intestinale, étant pratiquement neutres en solution aqueuse; mais lorsque, dans les parties inférieures du grêle, ils sont transformés en ions ferriques et en complexes organiques, ils irritent, grâce à leur pouvoir coagulant, les cellules du revêtement intestinal, et par là ils provoquent des symptômes d'intolérance. L'oxydation des ions ferreux en ions ferriques se fait dans le tube digestif, en milieu alcalin, c'est-à-dire à partir du duodénum. Il est vrai que les ions ferreux, dans la plupart des préparations se trouvant dans le commerce, sont stabilisés par la présence d'un acide (on emploie dans ce but surtout l'acide ascorbique). Cette stabilisation sous forme bivalente les protège contre la transformation en forme trivalente. Mais il ne fait pas de doute que bien des préparations soi-disant stabilisées

sous forme bivalente, ne le sont qu'imparfaitement. Et comme la forme ferreuse est très peu stable, le fer se transforme déjà dans l'emballage en sel trivalent.

D'autre part, lorsqu'on prescrit de trop fortes doses de fer, seule une partie en est résorbée; le reste provoque des symptômes d'intolérance en irritant la muqueuse intestinale. Il faudrait donc dans la mesure du possible, ne prescrire que la quantité résorbable. Dans le but de déterminer la quantité de fer qui est véritablement résorbée par le tube digestif, Hahn, Bale, Lawrence et Whipple ont administré à des chiens en état d'hyposidérose des doses croissantes de fer sous forme radio-active. Ces expériences ont montré que plus la dose ingérée est petite, plus la proportion de fer résorbé est grande; elle est par exemple de 60 % lorsqu'il a été administré 1,2 mg et de 3,2 % quand la dose est de 115 mg. Hahn et ses collaborateurs arrivent à la conclusion que 20 à 30 mg par jour représentent la dose thérapeutique optimale pour le chien. A notre connaissance, les auteurs américains n'ont pas étendu à l'homme ce genre de recherches au moyen de fer radio-actif. De notre côté, nous avons abordé ce problème en procédant à plusieurs déterminations du taux du fer sérique au cours de la journée, tout en augmentant progressivement la dose de fer prescrite.¹ Nos résultats concordent avec ceux des auteurs américains: l'ascension de la sidérémie ne se fait pas en proportion directe de la quantité de fer administrée, mais la sidérémie plafonne quand les doses sont supérieures à 3 fois 80 mg par jour, c'est-à-dire lorsque la quantité est environ dix fois plus forte que celle que Hahn et ses collaborateurs ont trouvé pour le chien: ces chiffres correspondent à peu près à la différence de poids entre le chien et l'homme. On ne peut donc pas forcer la résorption du fer en dehors des limites physiologiques et l'augmenter indéfiniment en haussant la dose administrée.

Quant aux troubles de la résorption et de l'assimilation du fer qui peuvent être à l'origine des symptômes d'intolérance, il y a lieu de supposer que le fer ne se trouve pas sous forme bivalente ou qu'il a été prescrit à de trop fortes doses, ou encore qu'il n'est pas résorbé. Après avoir discuté les deux premiers points, examinons le troisième.

L'intensité de la résorption dépend en premier lieu des besoins de l'organisme en fer: quand ces besoins sont grands, la résorption

¹ G. Hemmeler: »Les principes du traitement par le fer.« *Helv. Med. Acta*, 13, 488-524, 1946.

de fer est intense, et lorsqu'il n'y a pas de carence en fer, elle est minime ou nulle. Il découle de ces faits qu'en prescrivant du fer à un patient qui n'est pas en état d'hyposidérose, le médicament ne sera pas résorbé et pourra provoquer des symptômes d'intolérance. Cependant, certains malades en état de carence assimilent mal le fer, même lorsqu'il est administré sous forme bivalente. Nous le voyons dans certains troubles de la régulation de la résorption, tels que la chlorose, l'insuffisance hypophysaire, ou lorsque le transit duodéno-jéjunal est trop rapide, par exemple après résection d'estomac, chez des sujets atteints d'entérite, ou encore à la suite d'une atteinte grave de la muqueuse intestinale (maladie de Biermer, entérite chronique, sprue).

C'est donc dans les états d'hyposidérose, dus à un trouble de la résorption du fer, que la thérapeutique martiale parentérale est indiquée.

2. Stimulation médullaire exercée par le fer injecté par voie intraveineuse.

Heilmeyer & Plötner ont montré, par une analyse rigoureuse de l'effet thérapeutique obtenu, que le fer injecté par voie parentérale exerce une action stimulante sur la fonction de la moelle osseuse, et provoque une néoformation de globules rouges plus importante que lorsque le fer est donné per os, fait qui se traduit par des «crises réticulocytaires» particulièrement élevées. En outre, à la suite d'injections intraveineuses, l'organisme doit être à même d'employer pour l'hématopoïèse, des stocks de fer auparavant inutilisés. En effet, dans trois cas, Heilmeyer & Plötner ont calculé que la quantité de fer apparue sous forme d'hémoglobine dans les érythrocytes néoformés dépassait la dose injectée: l'effet thérapeutique était donc supérieur à 100 %.

De nouveaux arguments en faveur d'une stimulation de l'érythropoïèse par des injections de fer ont été apportés par Vuilleumier, qui a constaté fréquemment une baisse de l'index colorimétrique au début d'un traitement intraveineux de fer. Ceci s'explique par le fait que la production des globules rouges est proportionnellement plus importante que celle de l'hémoglobine.

Enfin, Goetsch, Moore & Minnich ont injecté du fer par voie intraveineuse à des sujets en bonne santé, et constaté dans trois cas un effet stimulant indiscutable, se traduisant par une

ascension du nombre des réticulocytes jusqu'à 36 ‰. En outre, ces mêmes auteurs ont obtenu chez des patients atteints de diverses anémies par manque de fer, après une injection intraveineuse unique mais massive de fer, des crises réticulocytaires dépassant toujours 100 ‰ et allant jusqu'à 247 ‰.

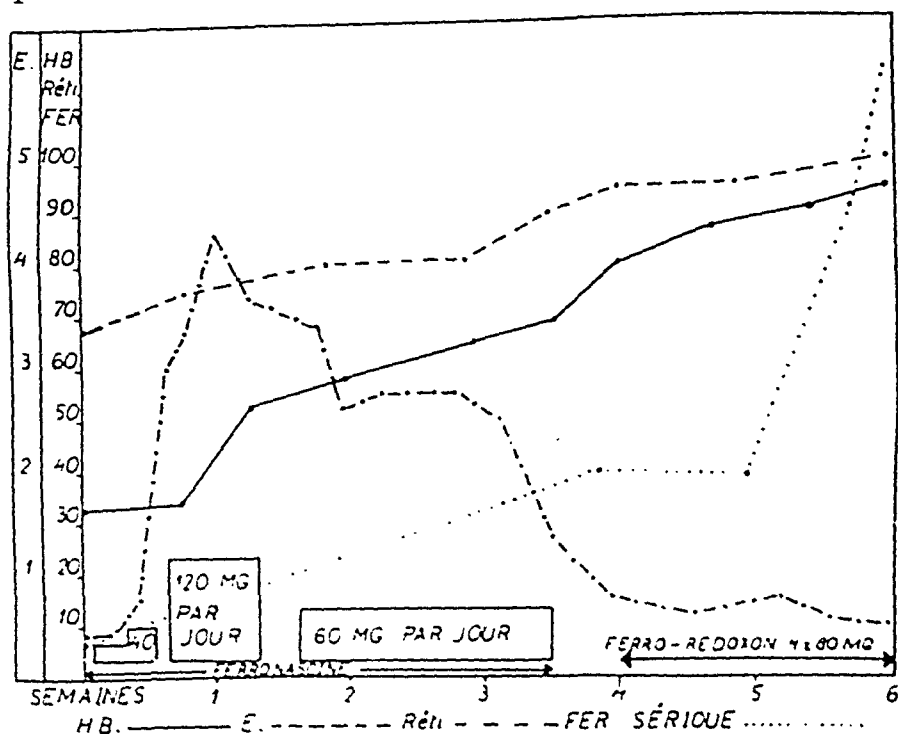


Fig. 1.

a) Homme de 31 ans, alcoolique invétéré qui présente une «anémie hypochrome essentielle». A l'entrée: Hgl. 31 ‰. E. 4,450,000, index 0.45, réticulocytes 9 ‰. Fer sérique 6 gamma‰. Suc gastrique normacide (après injection d'histamine, acidité libre jusqu'à 2 ‰, acide combiné jusqu'à 3,8 ‰). Pas d'hémorragie interne ou externe. La surcharge de fer per os montre une mauvaise résorption: 2 heures après l'ingestion de 120 mg de fer bivalent, la sidérémie ne dépasse le point de départ que de 44 gamma ‰. Il s'agit donc dans ce cas d'une anémie par manque de fer, consécutive à un trouble de la résorption.

Sous l'influence du traitement martial *intraveineux*, l'on assiste à l'apparition d'une crise réticulocytaire et à une rapide ascension du nombre des globules rouges et du taux de l'hémoglobine.

b) Homme de 48 ans, atteint d'une cirrhose hépatique avec varices oesophagiennes, dont la rupture provoque des hématomés et une anémie importante. Celle-ci ne montre pas de tendance à se compenser spontanément, les dépôts de fer étant épuisés (sidérémie 15

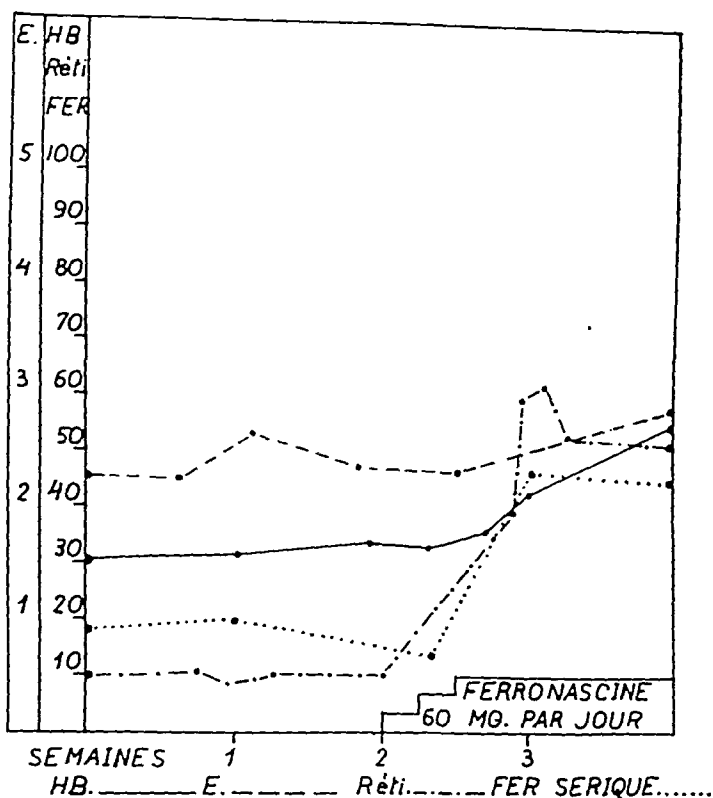


Fig. 2.

gamma%). Après une observation de deux semaines, début du traitement par 20 mg, puis 40 et enfin 60 mg *endoveineux* sous forme de Ferronascine. L'érythropoïèse reprend d'une façon accélérée, le nombre des réticulocytes atteint un maximum de 66 ‰, courbe ascendante du taux de l'hémoglobine et des globules rouges.

c) Jeune femme de 31 ans, souffrant d'une anémie consécutive à des métrorragies importantes, épuisant peu à peu ses réserves de fer. A l'entrée: sidérémie 17 gamma%. A la suite de la thérapeutique *intraveineuse* par la Ferronascine, ascension du nombre des réticulocytes et amélioration rapide de l'anémie.

Les exemples des figures 1 à 3 montrent l'ascension importante et durable du nombre des réticulocytes au cours de la thérapeutique *intraveineuse* par le fer. De tels chiffres ne sont pas atteints quand le médicament est donné per os.

Nous pouvons conclure de ces faits que le fer donné par voie intraveineuse exerce un effet stimulateur sur l'érythropoïèse plus grand que celui obtenu par la médication perorale.

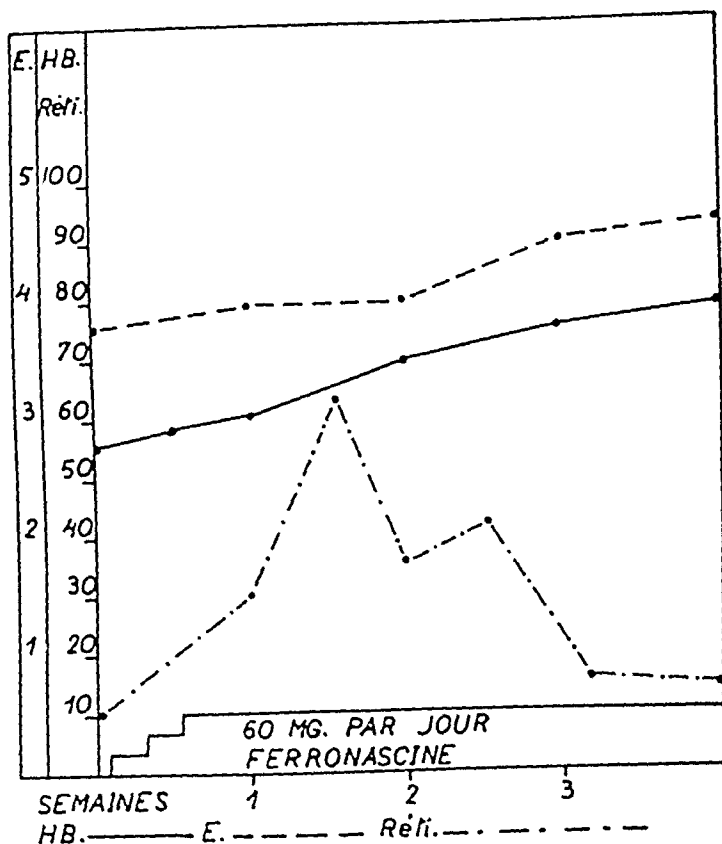


Fig. 3.

3. Forme chimique et posologie des préparations injectables.

L'effet thérapeutique est le même, que l'on injecte le fer sous forme de sel bivalent ou trivalent. Il est vrai que Vannotti (1) estime que les sels ferreux auraient une action surtout tissulaire, alors que leur effet sur l'hématopoïèse serait relativement limitée. En revanche, les produits à base de fer trivalent auraient une action surtout hématopoïétique, participant d'une façon particulièrement active au métabolisme du pigment sanguin. Pourtant, les résultats expérimentaux obtenus chez le rat anémié par Rothlin et Undritz, de même que ceux enregistrés par Mamie et Kalbermatten chez des sujets anémiques au moyen d'un traitement par le lactobionate ferreux (Ferro-Calcium Sandoz) ont montré que ce sel bivalent a un excellent effet sur l'hématopoïèse: l'anémie se compense rapidement. Par ailleurs, Dubach, Moore & Minnich

ont injecté du fer sous forme d'ascorbate ferreux à des patients souffrant d'anémie hypochrome: 10 jours après l'injection, ce fer se retrouve intégralement dans les globules rouges. Il a donc été entièrement utilisé pour la synthèse de l'hémoglobine.

D'autre part, l'effet thérapeutique des sels ferriques, par exemple le cacodylate de fer ou encore l'oxyde et l'hydroxyde ferrique colloïdal, est également bon. Ainsi, Goetsch, Moore & Minnich ont pu montrer que pour ces deux derniers médicaments, l'utilisation du fer injecté pour la néoformation de l'hémoglobine était de 71.8 à 99.7 %.

Il ressort donc de ces différentes expériences que l'effet thérapeutique ne dépend pas exclusivement de la forme chimique bi-ou trivalente du médicament employé.

Un autre problème à discuter est de savoir quelle est la forme chimique la mieux tolérée en injection intraveineuse. Etant donné que le fer alimentaire est transformé en FeCl_2 au niveau de l'estomac, grâce à l'action de l'acide chlorhydrique, on a pensé que c'est sous cette forme qu'il fallait injecter le fer, conception qui a donné naissance au «Ce ferro» chlorure ferreux stabilisé avec de l'acide ascorbique. Pourtant, le fer sous cette forme est relativement mal supporté par voie endoveineuse. On ne peut dépasser la dose de 5 à 10 mg par injection, sinon le malade se plaint de nausées, de maux de tête, de sensations de chaleur et d'évanouissement. Comme pour des raisons d'ordre pratique, il n'est guère possible de faire plus d'une injection par jour, une dose journalière de 5 à 10 mg s'avère être trop faible pour l'obtention d'un effet thérapeutique rapide. De fait, un simple calcul montrera les difficultés auxquelles on se heurte. Admettons que nous ayons à soigner une anémie de 50 % d'hémoglobine: la quantité de fer désirable pour compenser une pareille anémie est d'environ 1.3 g de fer, ce qui nécessiterait 130 injections de 10 mg chacune. Du reste, en indiquant la dose de 1.3 g nous n'avons en vue que la quantité destinée à couvrir le déficit en hémoglobine. Pour reconstituer les dépôts, épuisés en cas d'anémie hypochrome chronique, il faudrait en plus injecter environ 1 g de fer, ce qui impliquerait donc 100 nouvelles injections de 10 mg. La durée totale d'un tel traitement serait par conséquent de 8 mois environ: cette estimation est toute théorique, il est vrai. Elle ne tient pas compte de la résorption de fer alimentaire, qui a toujours lieu dans une certaine mesure.

Les sels de fer trivalents, tels que le chlorure ou le cacodylate

ferrique, pourraient être un peu mieux supportés; mais même sous cette forme il n'est guère possible de dépasser la dose de 10 à 20 mg de fer sans que des symptômes importants d'intolérance apparaissent.

Quant aux préparations à base d'oxyde ou d'hydroxyde ferrique colloïdal, nous n'avons pas d'expérience personnelle. De fortes doses de cette catégorie de médicament (jusqu'à 1.3 g) ont été injectées par Goetsch, Moore & Minnich à des patients atteints d'anémie par manque de fer, sous forme de goutte à goutte intra-veineux d'une durée de plusieurs heures. A la fin de pareilles injections, la sidérémie pouvait atteindre le taux extraordinairement élevé de 3,860 gamma %; en même temps, des symptômes d'intolérance grave se manifestaient. Evidemment, ces doses considérables avaient été injectées en une fois dans le but de provoquer une réaction hématopoïétique maximum. Les auteurs concèdent que les symptômes toxiques étaient tels qu'il est contre-indiqué d'utiliser d'aussi larges doses dans la thérapeutique martiale habituelle.

Les symptômes d'intolérance que l'on observe au moment de l'injection d'un sel ferreux ou ferrique sont dus au fait que le fer bi- ou trivalent se trouve sous forme ionisée. Si par contre le médicament est donné sous la forme d'un sel complexe dans lequel le fer n'est pas ionisé, l'injection ne provoque plus de symptômes secondaires. Mais comme l'efficacité du fer est liée à la nature ionisée, il est désirable que la ionisation se fasse dans l'organisme, c'est-à-dire graduellement dans les heures qui suivent l'injection; les ions peuvent alors se combiner avec les protéines du sérum sanguin au fur et à mesure de leur libération. C'est là la raison pour laquelle les phénomènes secondaires que l'on observe avec les sels de fer ionisé, ne se manifestent plus. Un des médicaments qui répondent à ces desiderata est la Ferronascine Roche. Il s'agit d'une préparation dans laquelle le fer se trouve sous forme de ferri-di-($\alpha\gamma$ -dioxy- $\beta\beta$ -diméthyl) butyrate de sodium. Les doses peuvent comporter jusqu'à 120 mg par injection (voir Fig. 1 à 3). Elles sont bien supportées. Il arrive exceptionnellement que les malades se plaignent de nausées, de sensation de chaleur et de céphalées dans les demi-heure à 2 heures qui suivent l'injection: c'est qu'à ce moment, semble-t-il, la libération des ions se fait à un rythme très rapide et que ces ions ne peuvent être liés en quantité suffisante par les protéines. De pareils troubles peuvent être évités si l'on se contente d'injecter 40 à 60 mg. Or, ce sont

déjà là des doses appréciables qui permettent de compenser un manque de fer de 1 g par exemple, en trois à quatre semaines à raison d'une injection par jour. De fait, l'effet thérapeutique peut être obtenu rapidement. Engel a publié des résultats qui correspondent à nos propres expériences, dont nous venons de donner trois exemples choisis parmi un grand nombre (voir Fig. 1 à 3).

En résumé, l'effet thérapeutique sur l'érythropoïèse (formation des globules rouges et synthèse de l'hémoglobine) ne dépend pas de la forme chimique sous laquelle le fer est injecté. Ce sont les ions ferreux ou ferriques qui provoquent les symptômes toxiques, aussi est-il avantageux d'injecter des sels de fer complexes dont les ions ne se détachent que graduellement à l'intérieur de l'organisme.

4. Evolution de la sidérémie après l'injection intraveineuse de fer.

La sidérémie, que nous avons dosée chez cent femmes et cent hommes en bonne santé, est en moyenne respectivement de 103 $\gamma\%$ et 132 $\gamma\%$. Un état d'hyposidérose est caractérisé par une chute marquée du fer sérique, qui peut atteindre un niveau très bas, jusqu'à 5 à 10 $\gamma\%$. Après une injection de fer intraveineuse, la sidérémie s'élève considérablement et peut dépasser 2,000 $\gamma\%$. (Dans l'expérience de Goetsch, Moore & Minnich, citée plus haut, elle atteint dans un cas 3,680 $\gamma\%$.) Pour autant que le fer en circulation ne se trouve pas sous une forme ionisée, ces sidérémies élevées sont non seulement bien supportées par les patients, mais encore ne provoquent aucun symptôme, si ce n'est, dans la majorité des cas, un goût de fer dans la bouche qui peut durer toute la journée. Ce symptôme n'est signalé qu'exceptionnellement tant que la quantité injectée ne dépasse pas 40 mg de fer (sel complexe).

Dans la Fig. 4, nous avons réuni un certain nombre de courbes de sidérémie dosée durant les vingt-quatre heures après l'injection de Ferronascine, chez des patients atteints d'anémie par manque de fer.

Les valeurs de départ sont de 5 à 10 $\gamma\%$; 10 heures après la surcharge, la sidérémie s'élève encore à 260, 621 et 650 $\gamma\%$, et même après vingt-quatre heures elle n'est pas encore revenue aux chiffres initiaux. Nous ajouterons que pour déterminer la sidérémie «à jeun» sans s'exposer à des erreurs, il y a lieu d'attendre quarante-

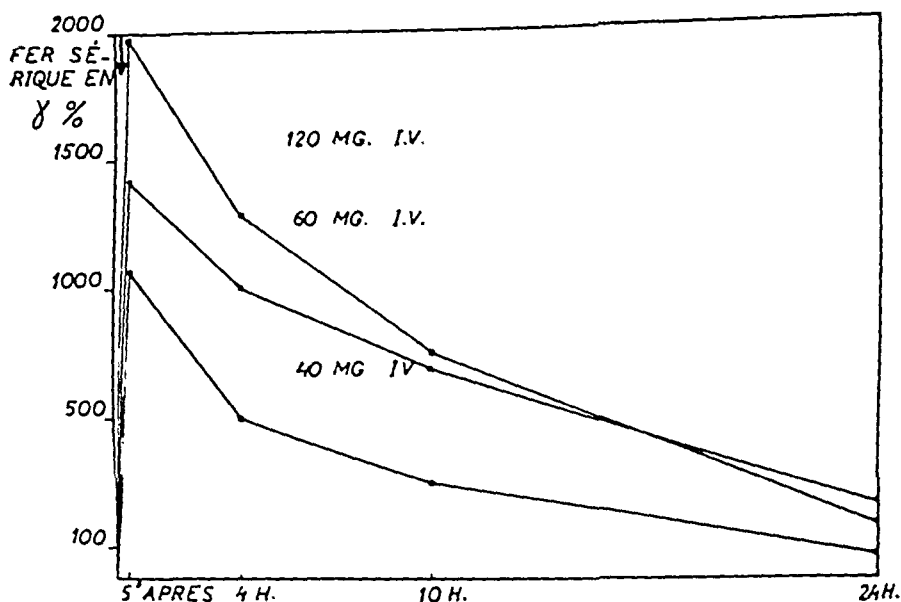


Fig. 4.

huit heures après la dernière injection intraveineuse: ce n'est qu'après ce laps de temps que la sidérémie est suffisamment stabilisée, ainsi que nous avons pu nous en rendre compte par des déterminations répétées.

L'expérience prouvant que le fer donné per os n'est pas résorbé lorsque le taux de fer sérique est élevé, nous pensons qu'il n'est pas indiqué de combiner le traitement intraveineux et le traitement peroral.

5. Précautions à prendre lors de la thérapeutique martiale intraveineuse.

Bien que nous n'ayons jamais eu d'accident grave à enregistrer, nous préférons ne pas employer les injections de fer par voie endoveineuse chez des personnes âgées. Ainsi que nous l'avons déjà dit, les médicaments à base de sels ferreux et ferriques peuvent provoquer des troubles circulatoires, qui se manifestent par des sensations de chaleur, des nausées et des vomissements. Objectivement, l'on constate d'abord une augmentation de la pression artérielle, qui est suivie le plus souvent d'une hypotension par vasodilatation. Avec un sel complexe, tel que la Ferronascine, de pareils troubles ne se manifestent qu'après injection de doses

supérieures à 40 mg. Il semble donc que les médicaments de cette catégorie peuvent être maniés avec une certaine libéralité; les doses habituelles sont au-dessous du seuil toxique, et la marge est assez grande pour qu'on puisse faire les ordonnances sans la crainte constante de dépasser la dose toxique.

Il y a un deuxième point sur lequel nous désirons nous étendre. C'est la question de la contre-indication des injections endoveineuses à un patient qui n'est pas en état d'hyposidérose.

Le métabolisme du fer est réglé de telle façon que l'organisme assimile du fer seulement lorsqu'il en a besoin. Il y a évidemment des exceptions, par exemple lors d'une surcharge avec des doses importantes, telles que 100 mg. Ce fait, qui a été démontré par Lintzel, a été confirmé au cours de ces dernières années par Hahn et ses collaborateurs, Moore et ses collaborateurs, Ross & Chapin, à l'aide du fer radioactif. Ces recherches particulièrement démonstratives ont prouvé que l'organisme oppose une barrière à la résorption du fer lorsqu'il n'en a pas besoin. En effet, alors que l'on supposait autrefois que l'excrétion du fer se faisait par l'urine et les selles, on sait aujourd'hui que l'organisme n'élimine des quantités notables de fer ni par l'urine, ni par la bile (Mc Cance & Widdowson, Hahn et collaborateurs, Vannotti (2)).

De fait, même lorsque l'on injecte de fortes quantités de fer par voie endoveineuse, l'élimination par l'urine et par la bile est très minime.

Nous avons injecté à un malade atteint d'une anémie hypochrome importante 120 mg de fer. L'urine a été prélevée 4, 8, 12, 16 et 24 heures après l'injection: son contenu en fer est minime et rapidement revient à la normale, qui est de 0 à 10 $\gamma\%$ environ. En tenant compte du volume d'urine excrété dans chacune des fractions, la quantité totale de fer éliminé n'est que de 1.5 mg. En ce qui concerne l'excrétion du fer par la bile, elle n'est que de 0.17 mg durant les premières heures après la surcharge. La sonde duodénale ne pouvant être laissée sur place plus de quelques heures, le dosage est incomplet, mais puisque l'élimination est peu importante au moment où la sidérémie est très élevée, il est peu probable qu'elle le devienne par la suite. Nous croyons donc être en droit de conclure qu'environ 2 % seulement des 120 mg injectés sont éliminés durant les premières 24 heures; dans les journées qui suivent, nous n'avons retrouvé que des traces de fer dans l'urine, résultats qui correspondent à ceux des auteurs américains obtenus au moyen de fer radio-actif.

Nous avons complété ces recherches par des expériences sur l'animal: trois lapins sains ont reçu en injection intraveineuses 5 mg de fer journellement, sous forme de Ferronascine, pendant

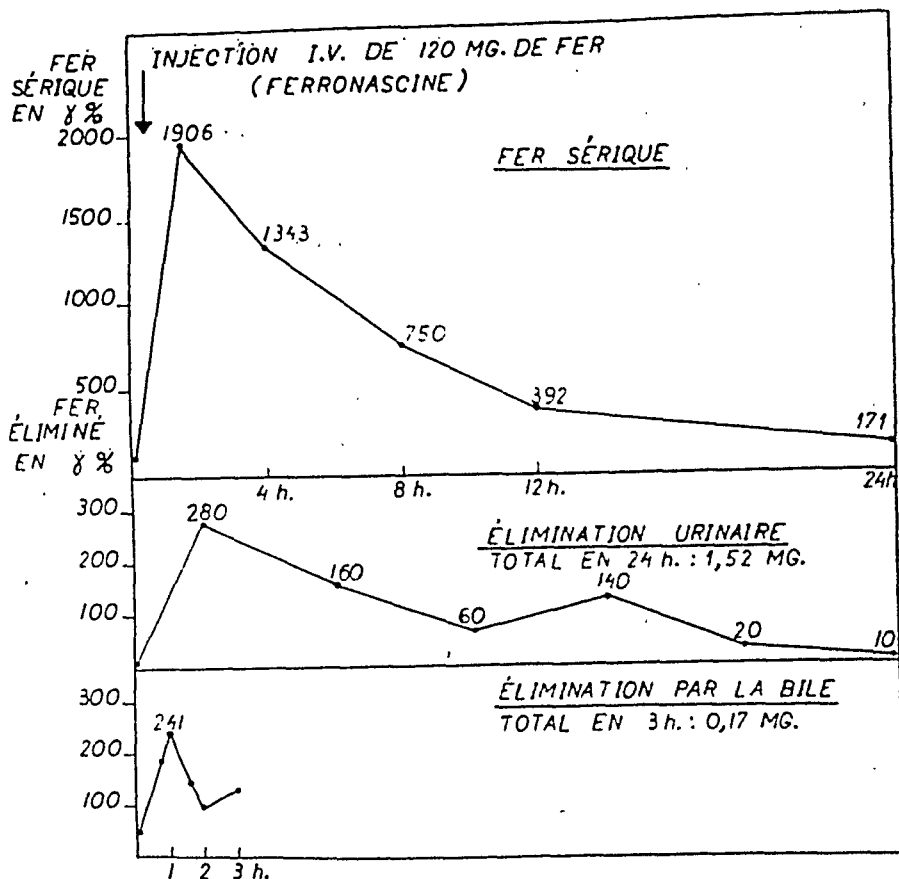


Fig. 5.

1 mois. Ces animaux ont été sacrifiés après 3 mois, et le fer a été dosé dans le foie et la rate, par la méthode à la phénanthroline, après calcination de fragment d'organe (environ 50 mg). Le foie a été perfusé avec de l'eau physiologique sitôt après son prélèvement. La rate, en revanche, n'a pas pu être perfusée, étant donné l'étroitesse des vaisseaux. Voici les résultats des deux séries d'examen.

Lapins-témoin.

	Poids du foie	Teneur totale en fer du foie
1)	90 g	15.23 mg
2)	72 g	14.70 mg
3)	93 g	25.17 mg
	Poids de la rate.	Teneur totale en fer de la rate.
1)	0.76 g	0.376 mg
2)	1.15 g	0.420 mg
3)	1.05 g	0.135 mg

Lapins ayant reçu 30 injections de 50 mg de fer chacune.

	<i>Poids du foie</i>	<i>Teneur totale en fer du foie.</i>
1)	95 g	43.75 mg
2)	105 g	52.05 mg
3)	79 g	39.48 mg

	<i>Poids de la rate</i>	<i>Teneur totale en fer de la rate.</i>
1)	1.90 g	10.05 mg
2)	2.2 g	9.04 mg
3)	1.6 g	12.51 mg

Ces expériences montrent que le foie des animaux ayant reçu des injections intraveineuses de Ferronascine, en tout 150 mg de Fe^{+++} , renferme en moyenne 2 à 3 fois plus de fer que celui des lapins témoins, et la rate environ 10 fois plus. En outre, en comparant la valeur absolue de la teneur en fer du foie et de la rate des animaux d'expérience avec celle des témoins, on peut calculer qu'environ les 2/3 du fer injecté ont été retenus dans ces deux organes.

L'ensemble des ces faits montre que non seulement l'organisme n'élimine pratiquement pas de fer à l'état normal, mais encore qu'il est incapable de se débarrasser du fer introduit par voie endoveineuse. Donc, la conclusion qui s'impose, c'est que si l'on prescrit à un patient du fer per os alors qu'il n'en a pas besoin, il n'en découle aucun inconvénient majeur. La résorption est barrée et l'organisme n'assimile pas de fer, ou du moins très peu, car il passe le tube digestif sans être utilisé. En revanche, si l'on injecte du fer à un sujet qui n'est pas en état d'hyposidérose, les choses se passent autrement: le fer n'étant pas utilisé pour la synthèse de l'hémoglobine, et ne pouvant pas être éliminé, il est stocké dans les organes de dépôt, notamment dans le foie et la rate. Cette thésaurisation artificielle et exagérée ne saurait que nuire au fonctionnement normal de ces organes.

Nous sommes d'avis par conséquent, qu'il est contre-indiqué d'injecter du fer à un patient qui ne souffre pas d'une carence en fer.

Summary.

The advantage of the intraveinuous iron therapy resides in the fact that erythropoiesis is stimulated in a more intensive way than when iron is taken per os. Moreover, it allows the treatment of

patients even when they suffer from a trouble of iron resorption, or when they do not tolerate enteric iron preparations. To obtain a quick therapeutic result, it is necessary to give iron in a form which, even at very high doses, may be well tolerated. This is not the case for ferrous and ferric salts. On the other hand, the iron-complex salts give excellent results in their effect upon the erythropoiesis, and symptoms of intolerance do not appear. The body, being unable to eliminate the iron not needed for the haemoglobin synthesis and for the re-establishment of its stocks, it is counter-indicated to prescribe iron for a patient not suffering from hyposiderosis.

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(Chief: Professor Sven Iugvar †.)

On Changes in the Organism Resulting from Insufficient Gas Exchange.

III. On the Cause of the Lowered Tissue Respiration in Insufficient Gas Exchange and on the Effect of High Carbon Dioxide Concentrations on Tissue Respiration.

By

HELGE COLLD AHL.

(Submitted for publication January 5, 1948.)

In insufficient gas exchange there are, besides the primary changes consisting of the lowered oxygen tension and the increased carbon dioxide tension, many other changes. In a previous paper some of these changes have been mentioned, *e. g.* the increase of the inorganic serum phosphorus content, the striking variations in the blood-sugar concentration, the increase of organic acids in the blood and the decrease of the bicarbonate concentration connected therewith. It may be questioned which of these different factors are of importance in reference to the lowered tissue respiration. Previously it has been shown (Colldahl 1943) that both the decreased oxygen tension and the increased carbon dioxide tension are important as to the lowered tissue oxidation in the liver. In the same paper it was shown that carbon dioxide tensions in the inspiratory air up to 10 % had no effect on the tissue respiration, if the oxygen concentration in the system was about normal. Only if the slightly increased carbon dioxide tension was connected with oxygen deficiency the effect could be seen. As acidosis ensues from oxygen deficiency it seems justified to ask if the factors which are influencing the tissue respiration

in the case of coexisting oxygen deficiency and carbon dioxide excess, form a combination of oxygen deficiency and carbon dioxide excess or a combination of increase of organic acids and carbon dioxide excess. In the latter case the lowered oxygen tension would have no direct effect of itself. Therefore it is of great interest to examine the tissue respiration in a state of acidosis, which should be as strong as in the experiments with decreased oxygen tension and increased carbon dioxide tension, but it is possible without coexisting oxygen deficiency. For instance, this is possible if the animals are given NH_4Cl . The tissue respiration can be examined in tissues from these animals when they have inspired ordinary air during the hours immediately before death as well as when they have inspired gas-mixtures with increased carbon dioxide concentration, e. g. carbogen (about 7% CO_2 , 90% O_2). In the latter case we have in the organism an increased carbon dioxide tension and a lowered bicarbonate concentration. On the other hand there is no oxygen deficiency as long as respiration and circulation are functioning satisfactorily. In insufficient gas exchange lowered oxygen tension will also be found. In the present paper the result of such a series of investigations will be shown. Experiments have also been carried out to investigate the effect of high carbon dioxide concentrations. Coexisting oxygen deficiency was avoided. Finally, histological experiments have been carried out on liver tissues of animals, exposed to insufficient gas exchange.

1. *The tissue respiration in acidosis through NH_4Cl*

The experiments were carried out on male guinea-pigs, weighing about 200 g. The animals were given a diet of beets, hay and crushed oats. With a pipette NH_4Cl was given per os in 3% solution. The first day the animals generally got $6 \text{ ml} \times 4 = 0.72 \text{ g}$, the second day $5 \text{ ml} \times 4$ etc. Smaller doses than 3 ml were not given. On the fourth-fifth days the animals showed increased respiratory frequency and looked unfit. In some cases the weight decreased considerably (in 5 days the decrease can be 25—30 g). The temperature of the guinea-pig often falls several degrees. The respiratory frequency which is first increased, falls at the same time as the animal's condition deteriorates. Also the heart frequency falls considerably. If the feeding is continued, the animals die. They lie down on their side and respiratory and heart

Table 1.

Date	Character of Exp.	CO ₂ combining power (vol. per cent)	Respiratory rate, when the animal was killed	Oxygen absorption in cmm after a given period in hours for the following tissues (200 mg wet weight):				
				Liver	Femoral muscle	Heart	Brain	Kidney
				$\frac{1}{2}$ 1	$\frac{1}{2}$ 1	$\frac{1}{2}$ 1	$\frac{1}{2}$	$\frac{1}{2}$ hrs
12. 9. 46	NH ₄ Cl + carbogen	15	16	119 228 128 241		61 110 30 55		120 124
7. 1. 47	NH ₄ Cl	12	10	111 180 105 181	122 186 131 191	31 47 32 66	68 74	91 110
7. 1. 47	NH ₄ Cl + carbogen	22	61	107 188 113 195	161 227 159 209	60 90 58 87	90 81	116 114
11. 1. 47	NH ₄ Cl	24	10	64 116 73 119	109 184 101 171	60 97 59 90		
3. 2. 47	NH ₄ Cl + carbogen	16	81	113 193 84 144	109 150 100 105	79 119 75 116		105 89
9. 2. 47	NH ₄ Cl + carbogen	16	8	103 169 87 147	151 188 143 169	29 48 39 58	73 70	102 116
12. 2. 47	Oxygen deficiency + CO ₂ excess	40	12	31 70 27 51	53 58 58 71	66 19 21 29	32 29	108
16. 2. 47	Normal ¹	74	100	68 100 70 95	163 324 152 290	34 50 32 52		
18. 2. 47	Oxygen deficiency + CO ₂ excess	26	48	51 14 14	148 136	35 37		
19. 2. 47	Oxygen deficiency + CO ₂ excess	28		43 37	79 70	145 125	251 188	9 19 21 33

frequency decrease more and more. If an animal with acidosis caused by NH₄Cl but in good condition otherwise, is exposed to carbogen, the animal gets worse during the next hour and dies, if the acidosis is strong enough. This must depend on the fact that through the inspiration of the carbogen the pH in the organism has become more acidotic. But in spite of the fact that the animals die in acidosis, there is no change in the tissue respiration as shown in table 1 (concerning the technique for tissue respiration see previous papers, Colldahl 1947). The result will be the same even in those cases *e. g.* the experiment $\frac{1}{2}$ 47) which must be expected to show in the end decreased oxygen tension in the animals in consequence of the continually decreasing respiratory

¹ More detailed reports of normal values are given in previous publications.

frequency, even if the high oxygen concentration of the carbogen counteracts the same during a very long time. This is, however, in accordance with previous papers where it has been shown that there will be no lowered tissue respiration if the animals die after having been exposed to oxygen deficiency only during a short time. The experiments recorded in table 1 illustrate the finding that, using this method, there will not be any lowered tissue respiration, if the animals die from acidosis only and oxygen deficiency is not coexisting as a dominating and long existing factor. In a following paper investigations will be made to estimate the phosphorus concentration in serum from animals which have been killed according to the above mentioned conditions.

2. Tissue respiration after high carbon dioxide concentrations in respiratory air.

In 1943 it was possible to show that it is easier to get lowered tissue respiration in the liver if lowered oxygen tension is combined with increased carbon dioxide tension. At that time no carbon dioxide concentrations higher than about 5—10 % were used. In this paper the effect of high carbon dioxide concentrations without coexisting oxygen deficiency has been investigated. When the carbon dioxide concentration in the respiratory air exceeds 20—25 %, the animals become narcotized (see Albitzky, 1912). As illustrated in table 2 a lowered tissue respiration is the result of these carbon dioxide concentrations. In the case of lower carbon dioxide concentrations which have no narcotizing effect and where the respiratory frequency is not lowered, the tissue respiration is normal. It is of considerable interest to point out that there is a great increase of inorganic phosphorus in the blood in the case of narcotizing carbon dioxide concentrations. As has been shown in a previous paper, the decreased tissue respiration in insufficient gas exchange might be parallel to phosphorus increase in the blood.

3. Histology of the liver after insufficient gas exchange.

Previously it has been shown (Colldahl 1943) that after insufficient gas exchange some enzyme systems are uninjured, while others are injured. Thus the succinate oxidation goes on normally, and it might be possible to draw the conclusion that the cytochrom system is more or less intact. This fact appears to

Table 2.

Date	Character of Exp.	Composition of gas mixture		Respira- tory rate when the animal was killed	Inor- ganic P in serum (mg %)	Oxygen absorption in cmm after a given period in hours for the following tissues (200 mg wet weight)									
		O ₂	CO ₂ (%)			Liver		Femo- ral muscle		Heart		Brain 1/2	Kidney 1/2 hrs		
						1/2	1	1/2	1	1/2	1				
17. 4. 46	CO ₂ excess	33	15	120	5.7	87 85	153 149	164 153	275 209	45 51	72 77	96 101			
17. 6. 46	Normal	ordinary air		100		114	225								
19. 6. 46	CO ₂ excess	36	31	12	23.0	29 31	53 57	105 119	156 175			43 61	98 93		
29. 6. 46	CO ₂ excess	25	25	16	19.5	32 31	59 57	152 176	221 256			75 77	117 108		

indicate that the cause of the lowered tissue respiration after insufficient gas exchange might not be a series of anatomical injuries in the tissues, *e. g.* as small necrosis. If that were the case, the result should have been a general enzyme damage as a consequence of the destruction of the tissue proteins in conjunction with the tissue destruction. Further, as previously has been shown (Coldahl 1943 a. 47) the lowered tissue respiration can be normalized by the addition of certain substances. This could not have been the case, if the cell proteins were damaged. It is, however, of interest to investigate the histological conditions in the liver after insufficient gas exchange. For that reason the tissue respiration has been investigated in a series of experiments and at the same time cuts of the same liver have been examined histologically. 10 cuts of each liver were examined.¹ The decrease of the tissue respiration of the liver in these experiments varied from a slight decrease to a large one. The microscopic picture of the liver was in all the cases normal at the time when the animal was killed. The animals were generally exposed to the changed gas tension during about 1½—3 hours. The normal histological picture of liver which shows highly decreased tissue oxidation in connection with the above mentioned results argue against an anatomical injury, causing the decreased tissue respiration.

¹ The histological preparations have most kindly been examined by Prof. G. G. Ahlström, Lund,

Summary.

In previous papers it has been shown that a lowered tissue respiration especially in the liver results from insufficient gas exchange, *e. g.* a diminished oxygen and a higher carbon dioxide tension.

In this paper it is shown that the same degree of acidosis which occurs in the experiments with insufficient gas exchange without diminished oxygen tension does not lower the tissue respiration. The lowered oxygen tension therefore seems to be of essential importance for the production of the decreased tissue respiration in insufficient gas exchange.

Inhalation of carbon dioxide contents in narcotic concentration, however, produces a lowered tissue respiration, even if oxygen deficiency is avoided. The cause will be studied later.

No histological changes can be found in the liver from animals which have been exposed to insufficient gas exchange according to the above mentioned conditions.

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Menstrual Disorders in Pellagra.

By

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Little attention has been paid to menstrual disorders in pellagra. Scanty menstruation is mentioned by Bicknell & Prescott (3). Menorrhagia and amenorrhoea are noticed by Goldberger & Sebrell (19). In the newer monographs by Taylor & Cayer (41) and by S. Harris (25), however, no reference is made to it.

This lack of interest may be due to the fact, that amenorrhoea and oligomenorrhoea were attributed to the poor general condition of the patients, since they also occur in many other wasting diseases. This, however, does not apply to menorrhagia and metrorrhagia which cannot be attributed to a poor state of health.

In this paper an account of menstrual disorders in a series of pellagrins is presented, with special reference to the interrelationship between vitamins and sex hormones.

Clinical Observations.

The present investigation is based on 25 females out of a total of 48 cases of pellagra; in 14, manifestations of pellagra occurred after the menopause. In another 3 cases, included in the series, the past history was unreliable, as pellagra had affected the memory. Of the remaining 8, one case of severe acute pellagra with pronounced and rapidly progressing anaemia, high fever and fatal outcome was the only patient with normal periods — a rather surprising observation. The 7 other patients were suffering from menstrual

disorders lasting from 6 months to 8 years, viz. menorrhagia 3 cases, metrorrhagia 1 case, amenorrhoea 3 cases.¹ These findings are summarised in table I.

The menstrual disorders occurred at certain periods of time, separated by intervals of normal menstruation, in some cases concurrently with other manifestations of pellagra, in others quite independently from the general trend of the disease.

Comment.

Admittedly, the above clinical observations would gain further weight if supplemented by the following laboratory investigations:

- 1) biopsy of the vaginal mucosa and endometrium,
- 2) estimations of the urinary output of gonadotropic hormone, estrogen and 17-keto-steroids (with reference to pituitary, ovarian and cortico-adrenal activity),
- 3) estimation of the urinary output of creatine, creatinine and phosphorus (with reference to a possible B-deficiency).

Since it has been shown that sexual dysfunction induced in rats by vitamin B deficiency may be corrected by gonadotropic or gonadal hormones (Moore & Samuels (33), Marrian & Parkes (32)), the therapeutic trial of such preparations might throw further light on the relationship between these two disorders; this method, however, is hardly practicable in view of the delay in starting nutritional treatment which it would inevitably entail. In many instances the correlation between clinical and experimental observations is admittedly more or less hypothetical; this holds good particularly for the study of vitamins, for it is well known that different species vary widely in their manifestations of the same nutritional deficiency, just as identical manifestations may be found in different species as the result of different deficiencies. Moreover, the trend in nutritional research is primarily the study of isolated deficiencies, whereas in man nutritional disease is often the result of rather complicated multiple deficiencies. As far as pellagra is concerned, it can by no means be identified with nicotinic acid deficiency, but, according to the excellent definition of Sydenstricker (41) represents «a B group

¹ We have seen two more cases of pellagra, in which hysterectomy was performed for apparently intractable metrorrhagia; the relation of the disorder to pellagra was overlooked; in one patient the operation was followed by an acute relapse of pellagrous phenomena. These cases are not included in the present investigation, since the case-reports are not available.

avitaminosis in which lack of nicotinic acid is predominant but in which other vitamins are depleted to a level of physiologic inadequacy».

Protein deficiency is also mostly present, as shown by the nutritional history and by estimation of the plasma proteins; moreover, caloric undernutrition, precipitated by prolonged diarrhoea and shown by emaciation, and various other associated deficiency states presumably coexist. In this respect recent observations have demonstrated the important fact that the absence of a second nutritional factor can modify or even abolish the manifestations of the deficiency of the first nutritional factor. Lastly, no adequate data are available for any species concerning the influence on sexual function of nutritional disorders. In spite of all these limitations it is hoped that an analysis of those relations may be of some use.

In the present state of our knowledge sexual disturbances in nutritional deficiency can be attributed to three mechanisms:

- 1) failure of hormone production,
- 2) loss of responsiveness of the effector organ,
- 3) decrease of hormone destruction normally occurring in the organism (Hertz, 26).

Amenorrhoea: As early as in 1922 Evans & Bishop (11, 12, 13, 14) reported that rats become anestrus as a result of inanition or vitamin B deficiency and that dried yeast was curative; further studies showed, however, that in these experiments vitamin E deficiency was the decisive factor: Parkes (37) and Marrian & Parkes (32) pointed out that the anestrus induced by B deficiency may be due to failure of the anterior pituitary; Mulinos & associates (34, 35, 36) arrived at similar conclusions; by partial inanition these authors produced in rats a condition which they termed pseudohypophysectomy. Anestrus was also produced in rats by means of deficiency in thiamine and riboflavine (Coward & associates (9), and by riboflavin-deficiency alone in experiments of Warkany & Schaffenberger (45). Similar results were obtained by Drill & Burrill (10) by means of vitamin B deficiency; anestrus was shown to be due to pituitary insufficiency since it responded to gonadotropic hormone.

Amenorrhoea (with or without edema) has been often reported by german authors during the first world war (»Kriegsamenorrhoe«).

In a woman suffering from partial inanition the writer observed

Table I. Occurrence of Menstrual Disorders in Pellagrins.

1	2	3	4	5	6	7	8	9	10	11
Total Number	Males	Females	Females after Menopause	Females of child bearing age	Amenorrhoea	Menorrhagia	Metrorrhagia	6+7+8	Normal Menstruation	Unreliable history
48	23	25	14	11	3	1	3	7	1	3

Table II. Association of Menstrual Disorders with other Manifestations of Pellagra.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Number	Name	Age (years)	Menstrual Disorder	Duration of the Disorder (months)	Other Manifestations of Pellagra								
					Skin	Mouth	Diarrhoea	Psychical Disorder	Anaemia (microcytic)	Electrocardiographic changes	Hyper-sensitivity to Insulin	Progress of the disease	Remarks
1	Jeanne Sch.	30	Amenorrhoea	18	++	+	Ø	Ø	+	+	++	much improved	Amenorrhoea since delivery Pellagra since 6 months
2	Maria Gi.	40	Amenorrhoea	?	+	Ø	Ø	+++	0	+	0	improved	
3	Lucio	17	Amenorrhoea	6-8	++	++	++	Ø	Ø	not examined	++	cured	
4	Stella Po.	27	Menorrhagia & Metrorrhagia	36	+	+	Ø	Ø	Ø	0	++		
5	Fortunée Mo.	24	Menorrhagia	periodically for several years	+	+	Ø	+	+	0	++	cured	ill for several years.
6	Hélène Mo.	24	Menorrhagia	periodically for several years	+	+	Ø	+	+	0	++	cured	ill for several years; twin sister of No. 5
7	Rachel Be.	48	Menorrhagia	8 years	++	++	++	Ø	++	++	(+)	improved premature dismissal	
8	Sarine Be.	23	Menorrhagia and Dysmenorrhoea	12 years	++	++	++	+++	++	++	+		
9	Fortunée Ha.	17	Unreliable past-history	—	++	+	++	+++	Ø	+	not examined	+	Fever; probably amenorrhoea
10	Rachel Ch.	23	Normal menstruation	—	++	+	++	+++	++	+	++	+	high fever progressive microcytic anaemia
11	Bahia Ha.	40	Unreliable past-history	—	++	+	+	+++	Ø	+	++	improved	

a condition closely related to pituitary cachexia (Simmond's disease) or to the pseudohypophysectomy of Mulinos and his associates; it was characterised by cheilosis and marginal glossitis, cachexia, loss of hair and pubes, oedema of the legs, arterial hypotension, alternating diarrhoea and constipation, and persistent amenorrhoea without gross local changes. For several months the patient was treated with anterior pituitary extract without any effect, but responded well to nutritional management.

Experimental and clinical observations therefore tend to suggest that the amenorrhoea in pellagrins is due to deficiency of anterior pituitary hormone. It remains undecided, however, whether the insufficient production of gonadotropic hormone is the only factor responsible for the menstrual disturbances; possibly, the gonadotropic function of the adrenal cortex is also impaired (either primarily or as a result of pituitary dysfunction). It is noteworthy in this connexion that anatomical changes in the adrenals have often been described in pellagrins (Finotti & Tedeschi (15), Thannhauser (43, 44), Aschoff (1), Froboese & Thoma (16), Froboese (17), Herzenberg (27).

Menorrhagia & metrorrhagia: The occurrence of menorrhagia and metrorrhagia in pellagrins can be correlated with experimental findings much more easily than can amenorrhoea since similar disturbances occur in other diseases and the therapeutic test is conclusive. Much is owed in this respect to the studies of M. S. Biskind and his associates.

Some relevant observations may briefly be discussed.

B. Zondek (46) reported the inactivation of estrogen by slices of liver tissue; this was shown to take also place in the whole animal (Israel, Meranze & Johnstone (28), Golden & Sevringhaus (20)).

In this respect M. S. & G. R. Biskind's (4) finding is important that the inactivation of estrogen by liver is absent in rats deficient in vitamin B. This has been confirmed by several authors who also reported that deficiencies in thiamine and riboflavine were the effective factors (M. S. Biskind & Shelesnyak (5), Singer & assoc. (40), Segaloff & Segaloff (39)).

In clinical cases menorrhagia and metrorrhagia have been reported in diseases now known to be due to deficiency states, *e. g.* chlorosis and cirrhosis of the liver. M. S. Biskind collected the relevant facts and correlated them with experimental findings.

Furthermore, M. S. Biskind and associates, by a series of thera-

peutic trials, have convincingly demonstrated that menorrhagia (as well as cystic mastitis and premenstrual tension) can be brought about by vitamin B deficiency (6, 7).¹

Although patients with menstrual disorders are seen only occasionally by the internist, the writer was able to confirm these observations in several instances. The following two case reports may serve to illustrate this point:

Case 1. (P. B.). A spinster aged 34, moderately obese, had regular periods since the age of 14, lasting 6 days and occurring at intervals of 28 days. Of her own accord she put herself on a weight-reducing diet by taking only one meal a day which consisted of rice, maccaroni, bread, soup, some meat and fruit. After 2 months, at the time of the expected period, a violent hemorrhage occurred; she was taken to a hospital on the fourth day. In spite of local and general hemostatic and hormone treatment the hemorrhage continued. On the 8th day injections of vitamin B complex were started on the writer's advice and after 48 hours the metrorrhagia stopped. Since then she has had 5 normal periods.

Case 2. (S. B.) A married woman, aged 39, had regular periods since the age of 15, lasting 8 days and being profuse for the first 3 days. She had two normal confinements. For a long time she had been on a restricted dietetic regimen in order to prevent increase in weight. She complained of increasing irritability, insomnia, loss of appetite and (on direct questioning) of a burning sensation in the feet, especially at night. On examination, there was conjunctivitis, perlèche, cheilitis, moderate marginal glossitis, brisk knee jerks without clonus, and tenderness of the calves; there was no disturbance of sensation. After a fortnight's nutritional treatment all manifestations had disappeared. Continuing the dietetic treatment for several more months the patient reported later that the duration of the periods had become reduced from 8 to 5 days and that the amount of blood loss had decreased.

These experimental and clinical observations leave little, if any, doubt that menorrhagia and metrorrhagia in pellagrins results from insufficient inactivation of estrogen due to functional liver damage of nutritional origin.

Damage of the liver is a constant anatomical finding in pellagra, especially in infants (Gilman & Gilman, 18) but also in adults.

¹ However, the possibility must be considered, that the functional liver damage is due to vitamin-B-deficiency only indirectly, by interference with the food intake. This relation is suggested by the experiments of Drill & Pfeiffer (Drill, V. A. & Pfeiffer, C. A., *Endocrinology* 38, 300, 1946), who found that paired-feed rats with B-vitamins lost the power to destroy estrogen like the B-deficient animals. Moreover, observations by György (György, P., *Proc. Soc. Exp. Biol. Med.* 60, 344, 1945) showed, that nutritional liver damage produced by protein deficiency prevented estrogen inactivation, which could be restored by the administration of lipotropic factors.

Deficiency of choline and protein, two factors known through recent work to play a part in producing liver damage (Best & assoc. (2), Patek jr. (38), Mc Henry & assoc. (29), Griffith (21), György & Goldblatt (22, 23, 24)), are not less prevalent in pellagra than are nicotinic acid deficiency and depletion of other components of the vitamin B complex (thiamine, riboflavine, folic acid). Hypersensitivity to insulin, also a regular finding in pellagra (Mainzer 30, 31), is possibly also due to this impairment.

According to the present state of our knowledge the above factors seem to be operative in the mechanism of the menstrual disorders in pellagra; a direct influence upon the ovaries of the deficiency state is a further possibility, not yet sufficiently investigated.

Summary.

Amongst 11 women of childbearing age, suffering from pellagra, menstruation was normal in only one patient. Amenorrhea was present in 3, menorrhagia or metrorrhagia in 4 and in the remaining 3 patients a reliable history could not be obtained.

Experimental and clinical observations have shown that of the organs concerned with the sexual cycle the function of the anterior pituitary, the adrenals and the liver are impaired by vitamin B deficiency.

There is evidence strongly pointing to a pituitary origin of the amenorrhea in pellagra. Arguments are put forward supporting the view that the menorrhagia and metrorrhagia in pellagra are due to insufficient inactivation of estrogen resulting from impaired liver function.

The rôle of adrenal damage present in pellagra and of ovarian failure induced by a hypothetical direct influence of the deficiency state is discussed.

Further investigations in pellagrins by means of biopsy of vaginal mucosa and endometrium and by estimation of the urinary output of gonadotropic hormone, estrogen, 17-keto-steroids, creatinine, creatine and phosphorus are suggested.

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On the Artificial Kidney IV. The Technique in Animal Experiments.

By

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Literary Survey.

In the years 1913—1914 Abel, Rowntree and Turner published the results of their experimental attempts to eliminate dialysable substances through extracorporeal dialysis of the blood of living animals. The aim was *partly* to study the substances which were diffused out from the blood in this manner, and *partly* to construct a therapeutically serviceable apparatus, namely an *artificial kidney*. The term was used under the express reservation that the mode of procedure in regard to the apparatus does not fully correspond to that of the kidney.

The apparatus was built of «celloidin tubes» which, at a cost of great labour, were made at the laboratory. A large number of tubes were necessary to achieve a satisfactory dialysis effect. For the largest construction not less than 192 such tubes were employed. The volume in an apparatus with 32 tubes varied between 800 and 500 ml blood, the dialysis area being about 3,200 cm². The largest appliance comprising 192 tubes had a capacity of 2½—3 litres of blood, and was intended for calves or animals of greater size. The tubes were built within a glass vessel of the Liebig condenser type. The blood was made to flow through the tubes, around which streamed saline solution. Obviously the content of blood in the apparatus was thus very great and consequently difficulties arose.

¹ Cooperation in heparin and narcotal experiments.

The blood was conducted from an artery, as a rule the arteria carotis, through the dialysis apparatus and back to a vein. Hirudine prevented coagulation. The blood-pressure forced the blood through the apparatus.

Tests were performed with dogs under chloretone narcosis. The celloidin tubes were filled with saline solution, as a rule, 0.6 % NaCl, and the apparatus was attached to the animal. The volume of the apparatus played an important part in the results of the experiment. According to the authors' calculations, the dog has 74 ml blood per kg body-weight. If the volume of the apparatus was so great as to correspond to 40 ml per kg of the experimental animal's weight, the dog would only be able to tolerate a short treatment, whereas if the volume was limited to about 30 ml per kg, the animal could live many hours.

After a while the blood-pressure began to fall, which was presumably due to the entrance of the saline solution from the blood paths into the tissues. The animal's blood volume was thus reduced in proportion to the amount which filled the apparatus. No attempt seems to have been made to have the apparatus filled with blood at the beginning of the test.

Two dogs which were only treated during 2 and 3 hours respectively, survived. With a longer treatment the animal died within 8—10 hours, one endured 16 hrs, another 18 hrs before they succumbed. Moreover it is stated that a rabbit out-lived a two-hours' dialysis treatment.

In a subsequent work (1914) the same authors study the various substances which were diffused from the blood into the saline solution. The dialysate collected from different animal tests during an interval of time embracing 112 hours, was investigated. Among the contents were 20 g non-protein nitrogen and, moreover, sugar, alanine, valine, histidine, creatinine, lactic acid, and so on.

Haas works with an apparatus which, excepting certain modifications, resembles that of the first named authors.

Between the years 1923—1928, Haas published short reports on dialytic treatment. His experiences are summarized in Abderhaldens Handbok, 1935. The following is a reference from the same.

The apparatus does not deviate in principle from the one described by Abel and collaborators. Hirudine was first used and then heparin against coagulation. The blood was made to flow through collodium tubes which, as a rule, seem to have had a diameter of 8 mm. An apparatus adjusted to a dog is said to hold 350 ml blood and has a dialysis area of 1,680 cm². As the apparatus was not filled with blood prior to its connection to the dog, a relatively great quantity of the animal's blood was lost to the apparatus.

The dialytic treatment of normal dogs could only proceed during the course of half, or at the most, $1\frac{1}{4}$ hours, on an average 30—45 min. if the animal should survive. The circulation was subsequently affected.

Haas was the first who attempted to treat patients with dialysis. These results are referred to in a later report concerning our clinical experiences of the dialysis method.

The first to treat uremic experimental animals, seems to have been H. Necheles (1923) and he also published his own construction of a dialyzer.

Necheles employed conically pointed tubes made of gold-beater's skin (peritoneum). The tubes were placed between two metal gitters; the content of the tubes was self-regulated, in other words, when the blood was forced into the tubes with high pressure, the gitter expanded and, by reason of its elasticity, collapsed when the pressure subsided. The apparatus consisted of ten such tubes joined together so that the dialysis surface was not less than 4,000 cm². The tubes were submerged in isotonic saline solution.

Necheles reported the details of dialytic treatment of a dog which two days previously had undergone double nephrectomy. Hirudine was administered to prevent coagulation. The blood was conducted from the arteria femoralis through the apparatus and back to the vena femoralis. During a dialysis of almost 3 hours the non-protein nitrogen in the blood sank from 122 to 101 mg% and the general condition of the animal was considerably improved. A renewed treatment, also of 3 hours' duration, the following day, gave a similar effect and the N.P.N. sank from 218 to 161 mg%. The two treatments resulted in the elimination of 5.4 g N.P.N. altogether. The dog died the next night.

Without further details it was stated that the results of several tests revealed »Durch zeitweise Dialyse wird das Leben des nephrektomierten Tieres nicht erheblich verlängert, dagegen wird ein Zurückgehen der urämischen Erscheinungen während und nach dem Versuche wahrgenommen, und es werden durch Dialyse aus dem strömenden Blute eine beträchtliche Menge stickstoffhaltiger Körper, überwiegend Harnstoff, entfernt«.

Without demonstrable inconvenience but with improvement of the uremic conditions it was thus possible to dialyze the operated dogs during 3 hrs. The treatment could also be repeated. These investigations seem not to have been continued.

Thalhimer, 1938, treated dogs which had been subjected to nephrectomy, with an apparatus built of cellophane tubes.

He wrote as follows. »In the experiments so far, from 1 to 4 of these tubes were used, each 2 cm in diameter and 30 cm in length. Through

a 2-hole rubber stopper tied into one end of the cellophane tube a glass tube was introduced to the bottom of the tube, the bottom end of the tube having been tied off with twine. Through the second hole in the stopper another glass tube, just long enough to penetrate the stopper leads the blood either back into the animal's vein or through the other cellophane tubes before returning it to the vein. The nephrectomized animal circulated his heparinized blood from his artery through the cellophane tube or tubes back into his vein. In from 3 to 5 hours 200—700 mg of urea nitrogen passed from the blood through the cellophane membrane into the surrounding physiological salt solution. No further details concerning the tests were given. Accordingly it is not possible to judge either the animal's reaction to the treatment or the effect of the latter on the nonprotein nitrogen of the dog. The report omits to mention whether the animal survived the treatment.

Prospective further communications regarding the method seem not to have been published.

Apparently even this author only performed a 3—5 hours' dialytic treatment. The yield seems relatively small and nothing is said about the effect of the treatment on the uremic condition.

Kolff and Berk, 1944, Kolff, 1946, constructed an apparatus which is discussed in another connection. Kolff performed no animal experiments, but applied his method direct to patients.¹

The preceding authors' apparatuses worked according to dialysis principles.

The dialysis and ultra-filtrate principles are combined in an earlier published modification of the dialysis apparatus employed by one of us in this paper, A. 1947.

It is possible, when necessary, by means of a simple siphonic device, to let the saline solution exercise strong negative pressure on the outer side of the cellophane tubing simultaneously as the blood exerts positive pressure on the inner side. In this manner fluid (ultra-filtrate) is removed from the blood. The elimination of retention-products occurs with this type of construction in accordance with the dialysis principles.

Malinow and Korzon, 1947, made a construction of an *ultrafiltrator*. By means of a pump or eventually, with the help of arterial pressure only, blood is conducted through a cellophane tube which is suspended in a vacuum chamber. A protein-free ultrafiltrate extrudes through

¹ *Addendum.* Muirhead and Vanatta (Am. J. Med., 1948. 3, 467): 'The application of the artificial kidney described by Kolff to nine nephrectomized and five normal dogs for one to three hours of dialysis resulted in death of the animals within a maximum period of forty-two hours, usually in less than twelve hours. These results indicate the technical difficulties in the procedure as described in the literature. The procedure, therefore, is not suitable for clinical application in its present status of development.'

the tube and is collected. In the treatment of animals this loss of liquid must be successively replaced with saline solution. The technique obviously becomes complicated.

The cellophane tube is attached to the arteria and vena femoralis of the anaesthetized dog. Heparin is the protective against coagulation. 7 normal dogs which were only treated between 1—5 hours survived. Of 4 nephrectomized animals, 2 expired after 2 and 3 hours' treatment, from loss of blood and lung-embolism respectively. The remaining 2 survived their 4 and 8 hours' treatment, but died of uremia 36 hours later. From the dog who underwent treatment 8 hours, 7,200 ml filtrate containing 6.9 g urea N. Urea N in the circulating blood sank from 180 to 75 mg%.

M. and K. conclude that the apparatus is not appropriate for therapeutic use by reason of its comparatively slight capacity. Moreover, the apparatus seems to be far too complicated for practical therapeutic employment.

Summary: Earlier dialysis treatment has almost unexceptionally been performed on dogs. The technique has generally been such that even normal dogs only survived for a few hours. Not more than two authors appear to have treated uremic dogs. The results of the treatment do not seem to have been of essential importance in lengthening the animal's life after nephrectomy. On the other hand, the technique usually involved considerable risks for the animal.

Our Results.

In order to elucidate the method's possibilities and risks we were of the opinion that a fundamental animal-experimental investigation was necessary as a preliminary step to therapeutic tests with uremic patients. Therefore the treatment had to be executed by means of a technique which would permit the animal to tolerate lengthy dialytic treatment. The apparatus must be of such capacity that the treatment is of therapeutic value in uremia. Here, an account is chiefly rendered of our positive results in respect to this work. Some brief information regarding certain difficulties we encountered would also seem of practical value.

Addendum: Recently G. Murray, E. Delorme and N. Thomas (Arch. surg. November 1947, 55, 505) described an artificial kidney. The cellophane tubing is, like ours, wound round a vertical wire mesh cylinder, submerged in salt solution. But, as their cylinder is devoid of a mantle, it was necessary to use cellophane tubing with a small diameter (0.63 cm) to reduce the amount of blood in the tubing. Consequently the dialysis capacity does not seem very great. A pump forces the blood through the apparatus. Dialysis treatment of dogs with slight uremia does not seem to have lasted more than 2—4 hours. During 8 hours' treatment of a slightly uremic patient (10 m tubing, blood-N.P.N. 120 mg%) 6.6 gm N.P.N. were removed.

Our experiments have been made with a dialyzer of earlier described type. The cellophane tubing is held compressed between two pieces of wire-netting, by which means the tubing can only contain a thin stream of blood and a limited quantity that is practically independent of the blood's pressure. The saline solution is moved by a motor-driven propeller.

With regard to our laboratory resources we have been obliged almost exclusively to use rabbits. The fact that these experimental animals are rather sensitive undoubtedly involved special difficulties but, on

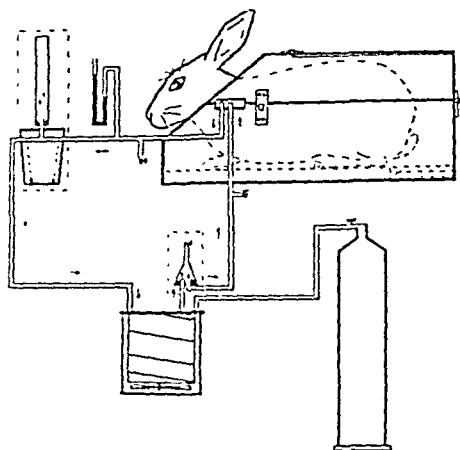


Fig. 1. The Fig. illustrates a schematic survey of the experimental arrangement. The details enclosed within a line of short dashes were excluded later. 1) The volume-meter furthest up to the left, and 2) the clot-air-trap which is seen attached to the dialysis apparatus. The simplified arrangement is as follows: The blood flows from the arteria carotis of the rabbit placed in the box, past the quicksilver manometer to the dialysis apparatus, and back to the vena jugularis. From the gas-bomb to the right of the dialyzer, oxygen-carbon-dioxide gas is bubbled through the saline solution.

the other hand, maybe, contributed in necessitating improvements in the technique.

Before passing on to details, reference is made to a survey of the experimental device reproduced in Fig. 1. Simplifications have been made at certain points during the course of the work. These are marked parenthetically.

The unanaesthetized animal is placed in a box. The rubber tubes lead to cannulae which, during local anaesthesia, were inserted in the arteria carotis and vena jugularis. The cellophane tubing of the dialyzer with pertaining tube conductors are filled with blood prior to its attachment to the animal. Heparin prevents coagulation. The arterial pressure drives the blood through the apparatus. A quicksilver manometer determines the pressure. (The flow of blood is regulated by means of a volume-meter. It passes a clot-air-trap on its way

from the apparatus to the vein.) The dialyser is placed lower than the experimental animal. The liquid balance, blood — saline solution, is regulated through adjusted hydrostatic pressure. The saline solution in the apparatus which is changed at suitable intervals is bubbled through with $\text{CO}_2\text{-O}_2$.

The Apparatus.

In the beginning we worked with a relatively long cellophane tubing in order to obtain a large dialytic area. This however demanded a great deal of blood to fill the tubing before the apparatus was connected with the animal. Consequently one or two rabbits must be killed in order to procure sufficient blood for the treatment.

If the treatment was executed without equipping the apparatus with the mantle of wire netting, mentioned in our earlier work, the cellophane tubing is liable to dilate under the influence of the blood pressure and take up a greater quantity of the experimental animal's blood, that is to say, the animal bleeds into the apparatus, the blood-pressure falls, eventually with deadly issue.

It had been apparent from the literature that bleeding into the apparatus was the usual experience with some authors who, for this reason, were obliged to limit their treatment to a few hours, if the animal should survive. The said bleeding occurred because the apparatus was not filled with blood prior to its connection to the animal. As previously mentioned, Kolff uses a long cellophane tubing which can take up great quantities of blood. If the balance between the influx and reflux is not maintained, risk is involved that the patient loses blood to the apparatus with the accompanying bleeding-shock.

Later on we reduced the length of the tubing to about 1.2 meters which corresponds to a dialysis area of somewhat more than 700 cm^2 . A flow of blood between $\frac{3}{4}$ and 1 litre per hour yielded excellent dialytic effect, as may be seen from the results published in an earlier paper, A. and N. 1947.

A considerable disadvantage, however, was that 60 ml of blood were requisite to entirely fill the apparatus. In order to acquire this quantity one animal had to be bled for each treatment.

The speed of the blood-flow was regulated by means of a Beek tube pump or a volume-meter described in an earlier paper. The apparatus was generally connected to the arteria and vena femoralis.

However, we found that without inconveniencing the animal the blood-flow could be increased to 2—3 litres per hour, and this, on the assumption that the blood be taken from the arteria carotis and returned to the vena jugularis. Consequently the exclusion

Table 1.

The Table illustrates the dialytic effect when 500 mg% urea solution flows through cellophane tubings 1.3 and 0.65 meters long respectively in the dialyzer for rabbits. Temperature 38° C. The results indicate the average of several tests performed in accordance with the technique described in an earlier paper.

Flow speed of urea solution. Litres per hr.	Total amount of urea conveyed to the apparatus. Mg per hour	Amount of urea dialyzed away per hour			
		1.3 m cellophane tubing		0.65 m cellophane tubing	
		Percentage	Total mg	Percentage	Total mg
0.5	250	64	160	51	128
1.0	500	48	240	36	180
1.5	750	38	285	27	203
2.0	1,000	31	310	22	220
2.5	1,250	26	325	18	225
3.0	1,500	—	—	16	240

of the above mentioned Beck tube pump or volume meter was possible. We were now able to reduce the cellophane tubing to 0.6 meters, the half, while still maintaining a sufficient dialytic effect. Accordingly, the content-capacity became only 30 ml, which amount can be tapped from an animal without risk of death.

Table 1 reveals: The dialytic effect with a 500 mg% urea solution, a cellophane tubing of (a) about 1.3 meters in length and a flow-speed of $\frac{3}{4}$ —1 litre per hour, and (b) a cellophane tubing of approx. 0.65 m in length and a flow-speed of 2—3 litres per hour is almost identical in either case.

In our earlier tests the blood was made to pass a clot-air-trap on its way from the apparatus to the vein. Later experiences showed however, the absence of risk of air emboli *if* the air be well evacuated before the tubing is connected with the test animal, and *if* the blood be allowed to flow from the animal through the apparatus in the direction, from above—downwards. In the practice of this direction, possible smaller air-bubbles in the tubing do not flow along with the blood, but remain in the tubing. Thus the cellophane tubing itself functions as air-trap. With reference to the hitherto administration of heparin, emboli have not been manifest through coagulation in the apparatus.

The experimental appliance illustrated in Fig. 1 has thus been simplified by the exclusion of the volume-meter and the air-trap. This simplification considerably facilitates the execution of animal tests.

The Narcosis Problem.

To begin with we used *urethane narcosis* for a rather long time. The animal was kept stretched out on an operation table. As a rule the blood was conducted from the *arteria femoralis* or *carotis* to the apparatus and back to the *vena femoralis* or *jugularis*. This was a convenient form of anaesthesia, suitable for our orientation experiments in respect to the effect of the dialysis on the test animal, and the effectivity of the technique as regards uremia. These tests generally lasted from 4—6 hours, but the narcotic state, at least one day more.

It was difficult to judge in the experiments as to whether the dialysis treatment tended to diminish the strength of the narcosis. The possibility of dialytic treatment in cases of soporific poisoning will be dealt with in another connection.

Then came the problem of keeping the uremic experimental animals alive for a longer time through repeated dialytic treatment several days in succession. The prolonged effect of urethane rendered this form of anaesthesia unsuitable. The animals did not become conscious between the treatments. It was obvious that narcosis was necessary in order to accomplish the tests and therefore another form of anaesthesia with an effect of shorter duration had to be found. *Ether* was considered to be out of the question owing to the risk of pneumonia.

We therefore tested *narcotal*, a drug for shorter periods of time which is used in surgical praxis (isopropyl- β -bromallyl-N-methylmalonylcarbamidnatrium).¹

The cannulae were inserted under deep *narcotal* narcosis which, latter, was successively administered intravenously. Afterwards, the anaesthetic strength was diminished so that the animal was always, almost conscious during the entire dialytic treatment lying stretched out on the operation table. If the animal showed signs of agitation, it was given more *narcotal*.

There now came a period of such difficulties that at times the achievement of a technique which rendered possible lengthy and recurrent dialysis treatment, seemed hopeless.

The animals died in numbers during, or in close connection with the dialytic treatment under decreasing blood pressure, as a rule decreasing content of plasma-protein, and not rarely fatal oedema of the lungs. The saline solution against which the dialysis took place nevertheless yielded negative proteinous reaction (sulphosalicylic acid). As a rule the breathing became superficial and frequent, the blood-sugar often revealed high values, in some cases over 800 mg%, and the bicarbonate in the plasma sank more or less rapidly to below 10 m.

¹ *Narcotal* was kindly placed at our disposition by the manufacturers, A.-B. Astra, Södertälje.

mol. Determinations of the total base of plasma yielded normal values. The hematocrite value generally decreased. It seemed possible in some cases, by means of insulin, to prevent the enhancement of blood sugar and the diminution of the alkaline reserves. Trial was also made with oxygen gas therapy.

In spite of an extensive work, with experimental variations we did not succeed in overcoming the difficulties. Among other things the administration of narcotal was limited to the time for inserting the cannulae. The treatment was performed with conscious animals placed in a box of the type illustrated by Fig. 1. Such being the situation it would seem that the following factors should be counted with as causal to the above difficulties.

1) The dialyzer. 2) The bleeding from the operation wound caused by the heparinization. It was impossible at this juncture to prevent the bleeding; attempts were made to compensate the latter by successive supplies of blood. 3) The narcosis.

The effect of the dialyzer was studied in the following manner:

The narcotal was administered to the animal in the same manner as for a dialytic treatment. On awakening from the narcosis the animal was connected with the apparatus reproduced in Fig. 1, the dialysis appliance being excluded. The blood consequently flowed from the arteria, past the quicksilver manometer, through the volume-meter and back to the vena jugularis. The speed of flow was limited to about $\frac{1}{2}$ litre.

Decrease of blood-pressure etc. also occurred even under these experimental conditions, and several animals died of lung oedema in more or less intimate connection with the test.

Under these circumstances it was necessary to more closely investigate the narcotal narcosis and to devote further attention to the operative technique with the object of reducing the bleeding tendency.

The aspects of our problem in respect to the *narcotal tests* were of a purely practical nature. The matter of importance was to prove to what extent narcotal might be a disturbing element in the dialytic experiment. For this reason we abandoned the idea of making a more thorough analysis and, instead, endeavoured to obtain answers to the following:

1) Can the narcosis be a direct cause of death relative to the experiment?

2) Does the narcosis contribute to the decrease of the plasma protein?

Table 2.
Plasma-protein of Normal Rabbits.

Rabbit Nr	Plasma protein, percentage				
	Initial value	Difference after hours			
		3	6	9	24
1.....	4.6	+ 0.2	—	+ 0.3	—
2.....	5.0	0.0	—	0.0	—
3.....	5.3	0.0	—	—0.1	—
4.....	5.3	—0.1	—	—0.1	—
5.....	5.5	—0.5	—0.5	—0.2	0.0
6.....	5.6	—0.3	—	—0.8	—
7.....	5.8	0.0	—	—0.3	—
8.....	5.8	—0.8	—	—1.2	—
9.....	5.8	—0.5	—0.5	—0.5	—0.3
10.....	5.8	—0.3	—0.1	—0.3	—0.1
11.....	5.8	—0.3	—0.3	—0.0	—0.1
12.....	6.0	—0.3	—	—0.2	—0.7
13.....	6.0	—0.7	0.0	—0.5	—0.2
14.....	6.0	—0.3	—0.5	—0.7	—0.2
15.....	6.0	—	—0.5	—0.7	—0.5
16.....	6.0	—0.8	—0.5	—0.5	0.0
17.....	6.2	—0.3	—	—0.2	—
18.....	6.2	—0.2	—0.9	—0.4	—0.4
19.....	6.3	—0.1	—	0.0	0.0
20.....	6.5	—0.2	—	—0.5	+ 0.7
21.....	6.5	—0.5	—	—1.0	—0.9
Difference, average	—	—0.30	—0.42	—0.38	—0.20

Rabbits were distributed in two groups so that the animals were about the same size in each group. The rabbits were removed from their ordinary hutches to the laboratory where they remained for the duration of the tests. This change of surroundings obviously affected the alimentation and so on, but the effect should have been similar for both groups. Blood samples were taken by puncturing the vein in the warmed, hyperemic ear. In the tables only the values of the total protein are given, determined at the beginning of the test (initial value) and after 3, 6, 9, and 24 hours, according to van Slyke's copper-sulphate method.

Table 2 reveals the changes in the plasma protein content of 21 control animals.

We refrained from analysing the blood otherwise, due especially to the advisability of not incurring any greater loss of blood through taking larger samples.

Under the present conditions the plasma protein lies, within the interval of 3—9 hours, lower than the initial value; mean values

Table 3.

*Plasma-protein of Rabbits, Anaesthetized with Narcotal
During 1—2 Hours.*

Rabbit Nr	Plasma protein, percentage				
	Initial value	Difference after hours			
		3	6	9	24
1.....	5.0	—0.2	—	—1.1	0.0
2.....	5.0	—0.5	—	—0.6	—
3.....	5.0	—	—	—1.4	— ¹
4.....	5.2	—0.7	—	—0.7	—
5.....	5.3	0.0	—0.1	0.0	+ 0.2
6.....	5.3	—0.2	—0.5	—0.7	—0.5
7.....	5.5	—0.3	—	—1.2	—0.5
8.....	5.7	—	—	—1.1	—
9.....	5.8	—0.5	—1.2	—1.2	—1.0
10.....	6.0	—0.5	—0.3	—0.2	—0.3
11.....	6.0	—0.3	—0.8	—1.2	—1.0
12.....	6.0	0.0	0.0	—0.5	—0.8
13.....	6.2	—0.5	—1.2	—0.9	—1.0
14.....	6.2	—0.2	—0.2	—0.2	—0.5
15.....	6.2	+ 0.3	—0.5	—1.2	—1.0
16.....	6.2	—0.5	—1.4	—1.4	— ¹
17.....	6.3	—0.1	—0.8	—0.8	—0.6
18.....	6.5	—1.0	—	—1.9	—
19.....	6.5	—1.3	—	—1.2	— ¹
20.....	6.6	+ 1.5	—0.8	—	— ¹
Difference, average	—	—0.28	—0.65	—0.92	—0.58

being — 0.30, — 0.42 and — 0.38 respectively. On the elapse of 24 hours the animals, as a rule, had still not regained the initial value, mean value being — 0.20. It should be emphasized that the small relative differences between the values after 3, 6 and 9 hours are not to be regarded as significant.

Table 3 shows the percentage of plasma protein of the animals treated with narcotal.

In this group an initial value was firstly determined. The animals were afterwards anaesthetized with narcotal intravenously for a space of 1—2 hours, and the strength of the narcosis was adjusted to approx. correspond to that which applied to the above dialysis test. From 5—10 ml narcotal was injected during the narcosis interval. The plasma protein was determined 3, 6, 9, and 24 hours respectively after the initial value. The value after 3 hours was accordingly determined a short time after the narcosis was ended.

¹ Died under the picture of pulmonary oedema.

As was the case with the control animals, we found a decrease of plasma protein during the observation period. After 3 hours the diminution occurred approximately at the same rate as that of the control animals. In the continuation the protein value sank still more, and conformably with the control material, was at its height after 9 hours. With regard to the lesser number of observations after 6 hours a comparison between the observations after 6 and 9 hours respectively was not possible; consequently there is no sure basis for the point of time when the minimum occurs. It is obvious, however, that the decrease of the plasma protein in the narcotal group after 6—9 hours is greater than in the control group, the average values in the former being -0.65 and -0.92 respectively against -0.42 and -0.38 respectively in the latter group. After 24 hours the decrease in the narcotal group is -0.58 against -0.20 in the control group.

It is accordingly established that under the present conditions narcotal anaesthesia reduces the plasma protein.

We also performed a series of tests to ascertain whether heparin tended to diminish the plasma protein. This series evinced no deviation from the control series.

Of the 20 rabbits included in the narcotal tests, not less than 4 died under the picture of lung oedema after 7, 12, 12, and 24 hours respectively. Thus, is demonstrated, that even a narcotal narcosis of such brief duration as was employed here, can endanger the life of the experimental animals.

The above investigation answered our questions and verified that narcotal anaesthesia is unsuitable for our dialysis tests.

We refrain from discussing here to what extent the said finds might be of more general interest.

Adriani, 1946, writes: »Data on the effect of anaesthesia alone on plasma protein are meagre. Stuart and Rourke, in studies of serum volume, report no change in plasma protein in man during ether anaesthesia. — — — anaesthesia with *amytal* and other *barbiturates*. — — — Apparently no gross deviation of globulin and albumin fractions occurs.»

Buck, 1941, found that ether and chloroform reduce the serum protein of the rabbit.

Moon, 1942, states that barbituric acids are liable to induce »spontaneous shock» to dogs, that is to say, the narcosis itself produces shock without simultaneous bleeding or other cause.

Gordh, 1945, reported the lung oedema of 3 rabbits in connection with vagal apnea, which was produced under deep narcotal narcosis.

When we, as will be seen in the following chapter, found, later, that it was possible to introduce the cannulae with local anaesthesia, and to keep the animal awake during the dialytic treatment while placed in a box, the narcosis problem became no longer actual to us.

The Operative Technique.

For a long time the operative intervention for inserting the cannulae was made in direct connection with the dialysis test.

By means of a skin-incision the artery and vein (*arteria femoralis* or *carotis*, *vena femoralis* or *jugularis*) were exposed, the cannula was inserted into the vein, the animal was heparinized, the cannula was introduced into the artery, and the wound was carefully sutured. Sometimes in the beginning it looked as if the blood had been effectively assuaged, but it soon began to trickle from the wound and had to be repeatedly replaced by small transfusions. It became especially difficult to compensate the bleeding when the experiments were prolonged over several days during the application of the arterio-venous anastomosis later spoken of, and dialysis treatment several days in succession. The sensitive experimental animals frequently died from loss of blood.

At first all the tried measures seemed in vain; from careful ligation and blood assuagement during the operation to several hours' pause between the operation and heparinization, the application of fibrin foam and cauterization, diminished dosage of heparin, up to risk for coagulation during the experiment.

The problem was solved later, however, in the following manner:

Two days prior to the dialysis treatment the animal's neck is depilated with the help of barium-sulphide. The irritation caused by the depilation has considerably lessened by the next day. On this day, the day before the treatment, an incision about 1.5 cm long is made under local anaesthesia, approx. over the *vena jugularis*, Fig. 2. The *arteria carotis* and *vena jugularis* are prepared as far as possible obtusely and made free from surrounding tissues. The lateral flap of skin is perforated in two places, the holes being intended for the insertion of the cannulae. A thread is put through the holes to guide the cannula when it shall be introduced through the wound the following day. Caudally of the holes some threads are drawn through the skin to be used later in fixing the cannulae to the same. A loose ligature is placed round the artery with which to draw the vessel forward when needed. No such thread should be put round the vein, however, as circulation impediments and thrombosis easily occur in the thin-walled vessel. Moreover the vein is not difficult to find at the following day's preparation.

The skin, in the middle of the wound, which latter has been treated with sulphamide, is then loosely sewn together.

In this way the wound lies half open, but closed to such an extent that the walls of the vein will be kept moist. In the wound lie threads which will be used the following day. The wound is covered with gauze into which the threads are folded. Plaster is used to fix the bandage.

The following day when the cannulae should be inserted, the animal is once more stretched out on the operation table. Local anaesthesia is injected in the skin. The one suture is taken away, whereupon the fibrin covered wound opens. The deep lying artery is carefully drawn

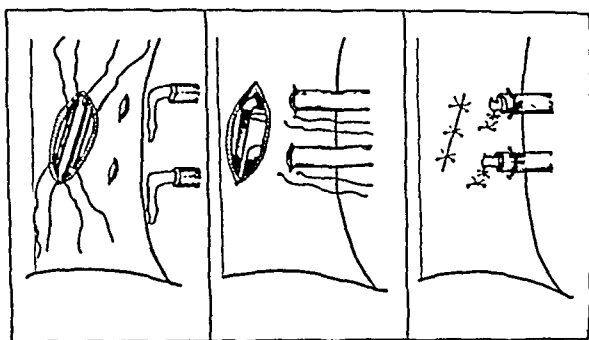


Fig. 2. The Fig. shows the operative technique and the cannulae. For further details see text.

forward by drawing the loose ligature already in position, as described above.

Guided by the threads and with the help of a blunt Péan's forceps, the small holes in the flap of skin are reopened. The cannula with adherent rubber tube, intended for the vein, filled with diluted heparin solution is introduced through the caudal of the two holes. The vein is tied cranially, the glass cannula is inserted and fixed with ligatures. The heparin is injected by means of the rubber tube, the dose being, as a rule 10 mg. The ends of the ligatures are now each tied to one of the threads which has lain, since the day before, stuck through the skin in connection with the small hole. The knots shall lie as near to the cannula as possible. The ends of the threads not in use are cut off. The cannula has now been attached to the threads which pass out through the skin. These threads are tied together, and in this way the cannula is fixed to the lateral wall of the wound.

The next step is the insertion of the arterial cannula through the small cranial hole in the skin into the artery. This cannula is fixed to the skin with the help of the earlier mentioned threads.

The preparation is now complete. Some sulpha-preparation is laid in the wound which is afterwards sutured with the said threads. The cannulae were thus introduced without piercing the wound or the skin. No bleeding occurs in the fibrin-covered wound surfaces either at the insertion of the cannulae or during the later dialytic treatment

with its prolonged heparinization. The preparation is made as sterile as possible and wound infection is rarely seen. The wound is covered with gauze and adhesive plaster.

The glass cannulae now pass through the small skin wounds while the big incision is well sutured. The animal can move its neck freely without hindering the circulation or damaging the vessels.

By means of this operation-technique the risk of bleeding from the operation-wound as a result of the heparinization has been practically eliminated.

The Coagulation Problem.

While the treatment goes on the animal is attached to the dialyzer by means of the rubber tubes of the vessel-cannulae. When the treatment for the day is ended and a repetition of the same will take place the next day, or if the treatment be otherwise interrupted, the following technique can be employed to keep the above cannulae permeable.

The rubber tubes are connected with a glass capillary which yields a flow of about 1 litre per hour. At intervals of 4 hrs, heparin is injected in the tubes which are wound round the animal's neck and fixed with plaster to prevent the animal from biting them. In this way the blood can continue to flow from the artery to the vein without coagulation.

Later we found that it was sufficient to fill the rubber tube, one end of which is closed and the other end connected to the cannula in the blood vessel, with concentrated heparin solution; in this way we consequently have no arterio-venous anastomosis, and coagulation in the cannulae can be prevented for a long time without heparinisation of the animal.

In extra-corporeal dialysis of the blood there exists a risk of coagulation which, without difficulty can be eliminated with heparin.

As published information, Jorpes 1946, indicates that heparin, even in large doses is not injurious to normal animals, we began with such big doses as 50—100 mg. Half was administered to the animal and the

Addendum to the proof: We found large retroperitoneal hemorrhages in some cases later, probably caused by ruptures when the rabbit was tied on the operation table and heparinized; for nothing of this kind has been seen in a control material. Therefore we have later employed brief ether anaesthesia at the insertion of the cannulas in order to avoid such rupture-bleedings.

remaining half was applied to the blood of the apparatus, before the experiment began. Such dosage neutralized the coagulative risk and was sufficient for a 4—6 hours' experiment. The bleeding from the operation wound involved difficulty as already mentioned, but this we learned to overcome gradually. Considerable bleeding occurred after injections for fluid or penicillin therapy. Such bleedings however could be avoided.

As reported in a later work there were moreover spontaneous bleedings from the back-muscles when the animal lay outstretched on the operation table, from the kidney and intestines in experimental mercurial poisonings, and in some cases even haemorrhagic pneumoniae. These causes of haemorrhage became increasingly actual when the treatments were repeated several days in succession and the animals were heparinized between the treatments too in order to maintain the circulation in the arterio-venous anastomosis.

With regard both to the animal experiments and to the possible bleeding risks in the event of treating patients, it became necessary to endeavour to avoid the bleeding danger without risking coagulation. This problem seemed to be of fundamental importance to the dialysis technique.

Our first step was to limit the heparin dosage with regard to the coagulation time, so that the latter was 25—30 min. at the most, half an hour after the last heparin dose. The heparin dosage was thus modified under continuous control of the coagulation time. In this way it was possible to reduce the dosage considerably. The bleeding risks remained however.

Our continued efforts to solve this problem have mainly been on the following lines:

1) Alkali-free glass for the cannulae and other necessary glass parts. Ordinary glass presumably induces coagulation more easily, as it gives off alkali.

2) Tests with paraffin treatment of the glass parts and connecting tubes. Unfortunately we have not observed any real progress in this respect.

3) Tests to uninterruptedly supply heparin to the blood just when it leaves the arterial cannula. We had hoped in this way, through lesser quantities of heparin to arrest the coagulative tendency during the passage of the blood through the apparatus without bleeding-risk for the animal. Hitherto it has been difficult to find the right measure of the heparin dose.

4) Tests to exclude heparin and employ sodium-citrate in the same manner as described in 3). Under continuous supply the body can eliminate large quantities of citrate without risk of being afflicted with cramp due to deionization of the blood calcium. The combination heparin—citrate is also being tested.

5) Tests omitting the supply of citrate direct to the blood but letting the blood, during its passage outside the body, flow through cellophane tubing which is surrounded on its outer side by citrate solution. This was technically solved as follows: a) The saline solution, against which the dialysis takes place, is supplied with a concentration sufficient to check the coagulation without riskely large quantities of citrate diffusing into the blood in the cellophane tubing. The last mentioned quantities are determined in tests with different flow-speeds. b) The blood is conducted to and from the apparatus through a narrow cellophane tube, encased in a rubber tube and filled with citrate solution. The practical arrangement will be described in another connection.

It should be pointed out that the presence of citrate in the saline solution entails no risk if the cellophane tubing should break. Should such occur, the blood is pressed out into the saline solution because of the high pressure in the cellophane tubing.

In connection with the description of the apparatus used here it must be pointed out that the construction of the same is adapted to diminish the risks of coagulation: a relatively short cellophane tubing whose entire dialysis capacity is exploited and through which the blood is conducted in a thin layer without stagnation, absence of rotating couplings and other movable parts, short tubular connections between the experimental animals and apparatus. These advantages are especially apparent in the treatment of patients and, as regards comparison, reference is made to the apparatus constructed by Kolff with its rotating cylinder, rotating couplings, pump, long and only partly filled cellophane tubing, as well as the long connective conductions between patient and apparatus.

An Example of a Rabbit Experiment.

The various details have been discussed and motivated in this and earlier reports.

Tables 4 and 5 give an example of repeated treatments of the same normal rabbit, different days. The rabbit which was kept heparinized without interruption and which was connected with the apparatus every time by the same vessel-cannulae, without renewed preparation, survived the treatments and does not appear to be affected by the same.

Addendum. Experiments are being performed by preparing all glassware and rubber tubes with a silicone coating according to V. O. Björk, Acta Chir. Scand. 1948. Suppl. 157.

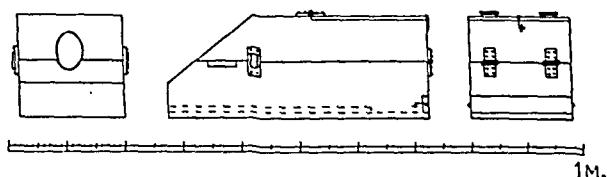


Fig. 3. The Fig. shows the size and construction of the wooden box in which the rabbit is placed during the experiment.

Table 4.

Dialysis Treatment of a Normal Rabbit.

Dialysis I, 29/11 1946			Dialysis II, 30/11 1946			Dialysis III, 2/12 1946		
Time	Blood pressure	Heparin mg	Time	Blood pressure	Heparin mg	Time	Blood pressure	Heparin mg
13.15	—	2.5	10.30	—	20	10.30	—	25
13.30	100	—	10.40	95	2.5	10.40	90	—
13.45	100	—	11.15	95	10	11.00	70	—
14.00	99	2.5	11.45	95	—	11.30	74	40
14.30	90	2.5	12.00	90	2.5	12.00	70	—
15.00	83	2.5	12.15	95	—	12.30	92	—
15.30	81	2.5	12.30	91	2.5	13.00	92	—
16.00	81	2.5	12.45	96	—	13.30	96	—
16.30	78	2.5	13.00	95	2.5	14.00	94	—
17.00	70	2.5	13.15	90	—	14.30	90	—
17.30	70	2.5	13.30	92	—	15.00	87	—
—	—	—	—	—	—	15.30	84	—
—	—	—	—	—	—	16.00	82	10
—	—	—	—	—	—	16.30	88	—

Table 5.

Changes in Blood Values During Dialysis of a Normal Rabbit.
(Table 4.)

Examination	Dialysis I		Dialysis II		Dialysis III	
	before	after	before	after	before	after
Hemoglobin, % ...	74	63	57	68	65	62
Red blood corpuscles, mill.	4.26	4.42	3.75	4.52	3.77	3.82
White blood corpuscles, mill.	6,400	11,100	6,800	11,500	4,200	6,700
Hematocrite, % ...	35	32	29	33	—	30
Blood sugar mg% ..	170	170	130	180	150	150
Plasma protein, % ..	5.0	4.6	5.2	6.0	5.7	5.5
Chloride, mg%	382	364	—	—	378	383
Total base, m. ekv .	148	151	—	—	148	149

Summary.

A detailed account of our own technique is given in connection with literature reports concerning earlier experiments in respect to extra corporeal dialysis treatment of animals.

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We are indebted to Lector Jens Bing, M. D., Copenhagen, for valuable consultations during the course of the work and to Professor Carl-Erik Quensel, Lund, for kindly helping us with the statistical problems.

Book Reviews.

The British Encyclopaedia of Medical Practice.

Medical Progress 1948. Editor-in-Chief, Rt. Hon. Lord Horder. London: Butterworth & Co. Ltd.; Africa: Butterworth & Co. Durban; Australia: Butterworth & Co., Sydney & Melbourne; Canada: Butterworth & Co., Toronto; New Zealand: Butterworth & Co., Wellington & Auckland.

The book is divided into 3 parts. Part I, Critical surveys, 168 pages; part II, Drugs, 10 pages, and part III, Abstracts of current literature, 333 pages.

At the end follows an index of 28 pages.

Part I begins with a chapter on medicine, written by Daniel Thomas Davies. Interesting topics are dealt with, for example: Penicillin in infective endocarditis, Toxicity of streptomycin, Vagal resection in peptic ulcer, Folic acid in sprue, Sympathectomy in hypertension, Thiouracil, isotopes in medicine, Psychosomatic medicine. Other chapters of special interest to the readers of *Acta Medica Scandinavica* are: Cardiology, by K. Shirley Smith; Chest diseases, by H. V. Morlock; Acute infectious diseases, by William Gunn; Aviation medicine, by Air Marshal Sir Harold Whittingham; Industrial medicine, by A. J. Amor; Psychological medicine, by A. A. W. Petrie and Dalton E. Sands; Tropical medicine, by Sir Harold Scott; Chemical pathology, by J. R. Mar rack; and Progress in vitamins, by Leslie J. Harris.

So far as can be seen, in all these very good critical surveys not a single paper is mentioned from the two hundred or so that *Acta Medica Scandinavica* has published during the past year. The same is the case with part III, Abstracts. It seems as if only a very restricted number of medical journals have been known and referred to for *Medical Progress, 1948*. This should be bettered.

I. Holmgren.

The Medical Annual, a year book of treatment and practitioner's index. Sixty-sixth year 1948. Bristol: John Wright & Sons Ltd.; London: Simpkin Marshall (1941) Ltd.; Baltimore: The Williams & Wilkins Co.; Toronto: The Macmillan Co. of Canada Ltd.; Melbourne: W. Ramsay; Sidney: Angus & Robertson Ltd.

As usual, the review of the year's work is written by a great number of the most prominent British physicians and surgeons and affords excellent information. In the editors' introduction special attention is paid to certain topics, for example the 1947 epidemic of poliomyelitis in England and Wales, mass immunization against diphtheria, DDT, protection against poisonous gases, B. A. L. and P. A. P. P., streptomycin, psychological medicine, thoracic surgery. On the question of B. C. G. vaccination the editors write: »More reliable evidence began to accumulate when the Scandinavian experts introduced first the subcutaneous and then the intracutaneous methods of administration and followed up the results with the usual care of the profession of their countries».

I. Holmgren.

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Further Investigations respecting Bilateral Hilar Adenitis.

By

HANS JACOB USTVEDT.

(Submitted for publication March 5, 1948.)

In a paper read at a meeting of the Internal Medical Society in Oslo in March 1939 respecting the nosography and diagnosis of Boeck's sarcoid (1) I stressed the significance of the fact that in Boeck's sarcoid hilar adenitis practically always presents itself radiologically as a bilateral hilar adenitis (B. H. A.), whereas as a component of the primary complex in tuberculosis it is most often found on X-ray examination to be present only on one side. I further emphasized the importance of bearing sarcoid in mind in cases of B. H. A., not only in tuberculin-negative, but also in tuberculin-positive patients, and I suggested the possibility of monosymptomatic forms of sarcoid, localized in the hilar glands. Finally, I drew attention to the frequent occurrence of joint affections in cases of B. H. A. in tuberculin-negatives, sometimes in combination with erythema nodosum (E. N.), and I touched upon the question of the place occupied by the articular phenomena in the clinical picture of sarcoid.

In the subsequent discussion on the subject J. H. Vogt (2) described a case of exanthema resembling E. nodosum, with protracted, bilateral H. A., in a tuberculin-negative patient, and he mentioned the possibility of sarcoid with E. nodosum or of Schumann's erythrodermic form of sarcoid.

In a publication in *Nordisk Medicin* the same year (3) I sub-

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mitted a material comprising 40 cases of B. H. A. in persons with negative or faintly positive tuberculin reaction. In 14 cases the cause was found to be general glandular tuberculosis, mycosis fungoides, malignant granulomatosis, lymphatic leucemia or general glandular diseases of uncertain nature (reticulo-endotheliosis?). In 13 cases there was found patho-anatomical or clinical evidence of the presence of Boeck's sarcoid, while the remaining 13 cases offered no etiological data. As regards the last-mentioned group of »non-specific» forms of B. H. A. I laid stress on the frequency of articular symptoms and pointed to the possibility of a relation to rheumatic fever on the one hand and to sarcoid on the other hand.

In his monograph on benign lymphogranulomatosis in 1942 P. B. Gravesen (4) reports a number of cases of hilar adenitis with miliary spread in the lungs, which clinically resembled in high degree the cases in which biopsy revealed benign lymphogranulomatosis. In at least one of Gravesen's cases the H. A. was bilateral. Gravesen also reports two cases of the triad E. N., H. A. and negative tuberculin reaction.

In 1943 L. Abrahamsen (5) reported four cases of the syndrome arthralgia, E. N., B. H. A. and tuberculin negativity. Abrahamsen surmises that the syndrome may have been occasioned by different infections as expression for an unspecific allergic reaction. In the same year Ask-Upmark (6) reported a similar case.

In 1945 N. Skiöld (7) published his large collection of cases of Erythema nodosum, in 35 of which B. H. A. was present. Bacteriological examinations were made in 19 of these cases, always with negative result. Neither were post-primary tuberculous manifestations noted in any of the cases. On the other hand, he found tuberculous manifestations during the further course of the disease in 30.5 per cent of the cases with unilateral H. A. Of 15 tuberculin-tested patients with B. H. A. only two showed negative reaction to Mantoux 1 mg, but the tuberculin sensitivity was in general slight. The great majority of the patients with B. H. A. belonged to older age-groups than the patients with unilateral H. A. In the 26 cases which were subjected to further radiological examination Skiöld found that the H. A. disappeared in the course of from 3 months to 3¼ years. None of the cases showed symptoms of Boeck's sarcoid. Skiöld seems most inclined to share Abrahamson's view that the majority of these cases have nothing to do with sarcoid. He lays weight especially on the fact

that in 10 out of 25 cases the H. A. had disappeared within the space of a year.

In his well authenticated work on E. nodosum from 1946 S. Löfgren (8) mentions 30 cases of B. H. A. among 178 cases of E. N., *i. e.* 16.9 per cent. In 20 of these cases parenchymal condensations were found in the lungs at the same time. Löfgren showed that, while the cases with B. H. A. formed only 8.4 per cent of the group with probable tuberculous etiology, they constituted 33.9 per cent of the cases in which a tuberculous etiology was improbable. But even in the group which reacted very strongly to tuberculin he found 7 cases of B. H. A. Löfgren's cases were distributed as follows among the different etiological groups: Tuberculous type 8.4 per cent, streptococcal type 6.7 per cent, other types 43.2 per cent. Within the last group Löfgren distinguishes between 1) a specially observed syndrome: E. N. with false positive sero-reactions for syphilis — no cases of B. H. A., 2) benign lymphogranulomatosis — 6 cases of B. H. A., and finally 3) what he calls the B. H. L. syndrome — 9 cases, as well as 4) four uncharacteristic cases.

Specially interesting is the fact that Löfgren does not find B. H. A. more frequently in streptococcal than in tuberculous E. N. By the B. H. L. syndrome he understands large, relatively symmetrical hilar lymphomas with negative or faintly positive tuberculin reaction. Löfgren cannot find any proofs to have been furnished that these cases bear any relation to benign lymphogranulomatosis.

In a work on tuberculin-negative cases of E. N. in 1946 J. H. Vogt (9) stated that his previously mentioned patient during further observation had presented no signs of Boeck's sarcoid and had shown negative Kveim reaction. In Vogt's 13 cases of probably tuberculous E. N. with H. A. the adenitis was always unilateral. Among the 7 tuberculin-negative cases there was one with B. H. A. and one, or possibly two, with unilateral H. A. Vogt suggests the possibility of a special syndrome, consisting of E. N., often with polyarthritides acuta, not seldom beginning with angina, occasionally accompanied by nephritis and not infrequently by unilateral or bilateral H. A., possibly of «rheumatic» etiology (streptococci?).

O. Forssman (10) reported in 1946 11 cases of E. N. and E. multiforme with negative tuberculin reaction, in 5 of which B. H. A. was present, and he discussed in this connection the

value of BCG inoculation ad modum Lemming for the diagnosis of L. b.

Isolated cases of B. H. A. have also been reported by Carl Müller (11), Bjerkelund (12), Kahrs (13) and others.

In an investigation respecting *E. exudativum multiforme* (14) I found unilateral H. A. in all the 36 cases with vesicular tuberculin reaction examined. Among 52 tuberculin-negative cases only one patient had H. A., and it was there bilateral, but without signs of Boeck's sarcoid.

In a material comprising 200 cases of *E. nodosum* (15) I found 84 cases of H. A., whereof 12 were bilateral, *i. e.*, 6 per cent of the whole material and 14 per cent of the cases of hilar adenitis. In 5 of the 12 cases a tuberculous primary infection probably existed. Among 69 cases of probably tuberculous H. A. five were bilateral, that is to say, 7 per cent.

The Material.

The diagnosis of hilar adenitis can be made only by means of radiography and is, as we all know, often difficult, even with the aid of Valsalva's experiment and of planigraphy. No cases in which there was the slightest doubt as to the diagnosis have been included in the present material. Where a typical picture of hilar adenitis with perihilar opacity was not found there has always been demanded distinct cyclic or polycyclic lateral delimitation of the shadow of the glands towards the lung-tissue. In the great majority of cases there was found *considerable* enlargement of the gland.

My material, which is derived from Med. Dept. A of the Rikshospital and from Dept. VIII and IX of Ullevaal Hospital, now comprises 77 cases, whereof over half have been under observation during 5 years or more and 16 during 10 years or more. Half of the patients were over 30 years and one-third over 40 years old, that is to say, a somewhat different age-distribution than in case of unilateral H. A. (cf. Skiöld). Erythema nodosum occurred together with B. H. A. in 27 of the 77 cases. Radiography revealed, besides B. H. A., also signs of miliary pulmonary condensations in 17 patients.

Without for the present adopting any standpoint as to the identity of Boecks' sarcoid with tuberculosis I have in the following account designated as Boeck's sarcoid those cases in which

histological examination revealed characteristic epitheloid cell granulomas without or with only quite insignificant central necrosis and without tubercle bacilli. By the designation tuberculosis I here mean to denote typical tuberculosis with distinct central necrosis in the granulomata, with possible presence of tubercle bacilli.

In 22 cases there were seen *simultaneous peripheral glandular swellings*, of which bioptical examination could be made. In a further 6 cases there were found, together with the hilar adenitis, *skin or eye affections* which permitted of bioptical examination. In the remaining 49 cases *no accessible material for biopsy was found*, apart from tonsils and nasal mucous membrane.

Table I.

Biopsy of Glands.

Tuberculosis	4
Hodgkin's disease	8
Lymphatic leucemia	2
Reticuloendotheliosis etc.	4
Boeck's sarcoid	4
	<hr/>
	22

Table 1 shows the results of biopsy in the 22 cases in the first group. Where peripheral glandular swellings are present at the same time as the hilar adenitis the diagnosis will in general be comparatively simple, and I shall not enter further into these cases. The four cases of tuberculosis embraced *two* typical cases of general, caseous glandular tuberculosis, combined in one of the patients with thyroideal tuberculosis, as well as one case of severe tuberculosis of the cervical glands and one case of chronic, benign miliary tuberculosis with negative tuberculin-reaction, where biopsy of a small gland from the fossa supraclavicularis confirmed the diagnosis. The patient is quite free from symptoms after an observation period of seven years.

Of the four patients with bioptically verified Boeck's sarcoid three have shown typical progression. The fourth is found after 10 years' observation to be clinically quite symptomfree, with normal lung picture and negative Kveim reaction.

The second group comprises 6 patients who had *affections of the skin or eyes* simultaneously with the adenitis. Five of these had exanthema, including four with typical Boeck's sarcoid. The fifth patient with exanthema was a woman aged 29, men-

tioned by R. Opsahl (16) in 1943, having B. H. A., signs of miliary spread in the lungs, E. nodosum, joint affections, weak tuberculin reactions and an exanthema which on biopsy showed resemblance to papulo-necrotic tuberculides. After 6 years' observation the patient is well, with normal lung picture and negative Kveim reaction. This case must be classified as tuberculosis. The sixth patient in this group had chronic irido-cyclitis with secondary glaucoma, and histological examination of an enucleated eye revealed Boeck's sarcoid.

The greatest interest attaches, however, to *the third group*, in which no material for biopsy was available and where the diagnosis is therefore far more difficult. The group embraces 49 cases.

Table 2.

Tuberculin Reactions.

Vesicular or strong (10 mm infiltration or more)	11
Moderate Pirquet-reaction (5—9 mm infiltration)	6
Weak tuberculin-sensitivity	12
	<hr/> 29
Negative tuberculin-reactions	20

Table 2 shows the results of tuberculin tests in these cases. In sharp contrast to what is seen in case of unilateral H. A. only a minority of the patients show great tuberculin sensitivity. No less than 32 out of 49 gave weak, variable or entirely negative tuberculin reactions. By faint tuberculin sensitivity is here meant Pirquet reactions with 2 to 4 mm infiltration, or negative Pirquet, but positive Mantoux 1 mg (at least 10 mm infiltration after 72 hours), or varying findings, but at least one certainly positive reaction.

Table 3.

	+++	++	+	÷	
Tuberculosis	6	1	1	1	9
Boeck's sarcoid	0	0	0	1	1
Boeck's sarcoid?	1	1	1	3	6
»Rheumatic type»	3	2	5	10	20
Respiratory affections	0	1	0	2	3
Uncharacteristic	1	1	5	3	10
	<hr/> 11	<hr/> 6	<hr/> 12	<hr/> 20	<hr/> 49

In Table 3 I have attempted to make an etiological classification. I wish to emphasize that this involves several elements of

uncertainty. I shall here give some account of the basis for this classification.

Tuberculosis. In the six cases with *great* tuberculin sensitivity (+ + +) we had to do with typical tuberculous primary infection, in four instances accompanied by E. N. In the one patient with *moderate* sensitivity (+ +) to tuberculin the subsequent occurrence of destructive pulmonary tuberculosis rendered it probable that the adenitis had been tuberculous. The one case with slight tuberculin sensitivity (+) is of special interest.

The patient was a woman aged 48, in whom Boeck's sarcoid was diagnosed on the basis of B. H. A., miliary condensations in the lungs, weak, sometimes negative tuberculin reactions and radiological observation of Jüngling's osteitis multiplex. Autopsy revealed, however, a typical miliary tuberculosis with necrotic foci and severe bilateral caseous tuberculosis of the salpinges. The clinical picture has been described by me in 1939 (1). The post-mortem findings will be dealt with in an article to be published in *Tubercle* (17).

The last patient with *negative* tuberculin reaction was a man aged 26 with severe affections of the joints. The glands afterwards perforated in the left main bronchus and tubercle bacilli were found in the sputum.

Boeck's sarcoid. Reliable evidence of the presence of Boeck's sarcoid has been found only in one single case in this group, namely, in a 25-year-old man with negative tuberculin reaction and positive Kveim reaction, where the affection of the lungs has shown the typical development through miliary condensations to fibrosis. Biopsy of the Kveim papule revealed characteristic epithelioid cell granulomata.

In addition there were 6 cases in which the possibility of the presence of sarcoid may be said to be more or less probable.

1) A patient with *great* tuberculin sensitivity, a woman aged 44, who in conjunction with a change from negative to highly positive tuberculin reaction showed B. H. A. with perihilar condensations. The patient reacted positively to tuberculin dilutions down to 1/100000. That the diagnosis Boeck's sarcoid came into consideration at all is due to the fact that Kveim's test, carried out at the Dermatological Dept. of the Rikshospital, gave positive result. Although tubercle bacilli could not be found in gastric lavage fluid, the case must be registered as primary tuberculosis, and the positive Kveim test must for the present be regarded as an unspecific reaction.

2) A patient with *moderate* tuberculin sensitivity, a man aged 34, with chronic bilateral irido-cyclitis, B. H. A. and miliary pulmonary condensations, which have shown increasing diffusion during 8 years.

The irido-cyclitis is of the type that may be seen in case of sarcoid (but also in tuberculosis). The lung affections followed a course similar to what is seen in cases of sarcoid. Here, however, Kveim's reaction was *negative*. It appears from Danbolt's works (19, 20) that Kveim's test seems on very rare occasions to give a negative result in typical Boeck's sarcoid, and possibly we have here to do with such a case.

3) Woman aged 53, with considerable B. H. A. and dense miliary condensations over both lungs. Biopsy from tonsils gave negative findings. Tuberculin tests negative. After an observation time of 8 years the patient is quite free from symptoms, but refuses to undergo X-ray examination or Kveim's test. Boeck's sarcoid?

4) Woman aged 25, tuberculin-negative, with recurrent E. nodosum and joint affections, B. H. A., but clear lungs. Kveim's reaction is negative. Cutaneous BCG inoculation has given positive result, and the papule showed tuberculous structure, but tuberculin positivity was not attained. The patient is under observation.

5) Woman aged 32, tuberculin-negative, with protracted B. H. A., without E. nodosum or joint-pains. Half a year after the beginning of the illness Kveim's reaction was positive, but is now, two years later, negative, while at the same time the glandular swelling has disappeared and the patient is quite free from symptoms. As to whether we have here to do with an unspecific Kveim reaction at the first examination or with a rapidly cured case of Boeck's sarcoid we are for the present without means of deciding.

6) A woman aged 28, faintly tuberculin-sensitive, with uveo-parotitis, peritonitis and B. H. A. (case described by Arne Mohn (18)). Ten years later there developed symptoms of bilateral pulmonary fibrosis with large cavities. Tubercle bacilli could not be detected. The patient died after attacks of profuse hemoptysis. At autopsy (cf. *Tubercle* 1948) no histological signs of tuberculosis or sarcoid were found, but only an enormous pulmonary fibrosis with cavities and unspecific inflammatory changes. The possibility exists that it is here a question of the final stage of sarcoid, where the specific inflammatory changes have been completely stifled by the fibrosis.

Of these six cases one (No. 1) must presumably be registered as tuberculosis, three (Nos. 2, 3 and 6) as possible Boeck's sarcoid, while two (Nos. 4 and 5) cannot as yet be classified. »Transitional cases» between typical sarcoid and ordinary tuberculosis are sometimes seen, and the adherents of the theory of the tuberculous genesis of the sarcoid find, in my opinion, their best support in such cases. Continued investigations respecting the specificity of the Kveim reaction will here be of great importance.

Uncharacteristic cases. In ten cases, including three with E. nodosum, no clinical peculiarities that could justify a classification were noted. Joint affections or symptoms from the air passages were not found in any of the patients in this group. The

situation as regards tuberculin sensitivity is shown in the table. Kveim's test was carried out in three of the patients, with negative result. Tubercle bacilli were not detected in any of them. In the seven patients who could be kept under observation the hilar adenitis disappeared in the course of 3 or 4 months.

Affections of the air passages. Transitory B. H. A. was observed in three cases during affections of the air passages, namely, in one case together with febris catarrhalis, in the second with bronchitis foetida, in the third with asthma bronchiale. The occurrence of H. A. in affections of the air passages is known from, for example, Kjellberg's work (19). It is in itself not unreasonable to suppose that such H. A. may be bilateral.

»*Rheumatic type*». In 20 patients the clinical picture has been characterized by *more or less severe joint affections*. Careful clinical examination has failed to reveal signs of tuberculosis or of Boeck's sarcoid in these patients. As the symptoms from the joints may possibly be taken to bind these cases together in one group, I have tentatively designated them as »rheumatic», but *with all possible reservations*.

Table 4.

»*Rheumatic Type*» without Signs of Tub. or Sarcoid (20 Pat.)

Acute polyarthritis	6
Chronic polyarthritis	1
Bechterew's disease	1
Uncharacteristic phenomena	12
Rheum. acutus earlier	2
Frequently sore throat	4
Scleritis	1

Table 4. In six of the cases the clinical picture accorded with that of a mild rheumatismus acutus, with pains, sometimes quite severe, occurring successively in several joints, with unquestionable swelling in one or more joints and with greatly increased S. R. In one case there existed a chronic rheumatic polyarthritis, in another case an affection resembling Bechterew's disease. The remaining 12 patients had joint pains of different intensity and localisation, without distinct swelling. As to the question of »rheumatic relations» it is of interest to note that two of the

patients had previously had acute rheumatism, four had often been troubled by angina and one had scleritis at the same time.

Table 5.

»Rheumatic Type» without Signs of Tub. or Sarcoid (20 Pat.)

Weak or neg. tub. reaction	15
Erythema nodosum	16
Miliary spread	4
Negative Kveim reaction	12
X-ray hands and feet neg.	11
Biopsy tons. neg.	7
Pathological Ekg.	1

Table 5. Fifteen patients showed negative or weak tuberculin reactions. Sixteen had at the same time E. nodosum or E. multiforme and in several cases both erythema and joint pains appeared recurrently during many months. Miliary condensations, transient and not very pronounced, were noted on X-ray examination in four cases. Kveim's test was made in 12 cases, in all of them with negative result. X-ray examination of hands and feet (11 cases) and biopsy of tonsils (7 cases) likewise gave negative results. Transient electrocardiographic changes (prolonged P—Q interval) were noted in one case (electrocardiograms were taken in all cases). The patients were for the most part females, and 14 out of the 20 patients were 30 years old or more. Twelve were under observation for from 6 to 12 years, and all of them proved to be free from symptoms, without signs of B. H. A., tuberculosis or Boeck's sarcoid. Four have afterwards had periodical joint troubles.

It might seem as if we had here a comparatively well-delimited group of cases, but the matter is not so simple as that. Exactly the same combination of B. H. A. with joint affections, E. nodosum and faint or negative tuberculin sensitivity may be seen, although seldom, both in bacteriologically verified tuberculosis and biotically verified sarcoid, and this applies also to cases in the present material. Thus one of my sarcoid cases began clinically with a typical acute polyarthritis. A case of chronic miliary tuberculosis, with a caseated gland in the F. supraclavicularis and negative tuberculin reaction, was attended by B. H. A. and pronounced painfulness of the joints.

The joint phenomena play, as is known, an important part both in E. nodosum and in E. multiforme, irrespective of the etiology of the cases. J. H. Vogt points out how the frequency of acute

polyarthrititis in cases of *E. nodosum* increases according as a tuberculous etiology becomes less probable. Löfgren reports joint pains in 46.2 per cent of the cases of *E. n.* due to tuberculosis, in 63.3 per cent of those caused by streptococci and in no less than 77.3 per cent of the cases which seem to be ascribable neither to tubercle bacilli nor to streptococci. Even if we conceive the joint troubles, including the acute polyarthrititis, in such cases as being an allergic reaction to various noxae, it is evident that cases due neither to tubercle bacilli nor to streptococci are especially characterized by such reactions. The present material shows that this holds good also for B. H. A.

Table 6.

Acute Polyarthrititis without Signs of Tuberculosis.

In BHA	6 of	77 cases
In <i>E. nodosum</i>	5 "	200 "
In <i>E. multiforme</i>	15 "	202 "
In atypical <i>E. mult.</i>	5 "	17 "

Acute Polyarthrititis with Signs of Tuberculosis.

In BHA	1 of	14 cases
In <i>E. nodosum</i>	2 "	132 "
In <i>E. multiforme</i>	4 "	35 "

Table 6 shows how a picture resembling acute rheumatism may appear in these different conditions, both with and without simultaneous signs of tuberculous disease. The figures are obtained from our own materials. It has further been shown by, among others, Dedichen (20), Heimbeck (21), Owren (22), and Ustvedt (23) that acute rheumatism without exanthema, sometimes with myopericarditis, may accompany the tuberculous primary infection.

On the basis of my own investigations (77 cases of B. H. A., 60 cases of unilateral H. A. without exanthema, 200 cases of *E. nodosum*, 202 cases of *E. multiforme* and 266 cases of acute rheumatism) I have in Table 7 sought to furnish a general survey of these complicated conditions.

B. H. A. with or without *E. nodosum* and joint phenomena may be seen both in tuberculosis and sarcoid and in other conditions. Unilateral H. A. without *E. nod.* or joint affections is typical of tuberculosis, is seen on rare occasions in cases of sarcoid

Table 7.

	Tub.	Not tub.	Boeck
BHA without joint phen. or E. N.	+	+	+
BHA with joint phen., without E. N.	+	+	+
BHA with joint phen. and E. N.	+	+	+
HA without joint phen. or E. N.	++	(+)	(+)
HA with joint phen., without E. N.	(+)	(+)	?
HA with joint phen. and E. N.	+	+	?
Rh. ac. without HA or E. N.	+	++	?
Rh. ac. without HA, but with E. N.	+	+	?
E. nod. without joint phen. or HA	+	+	?

and may accompany affections of the air passages. Unilateral H. A. with joint phenomena, but without E. nodosum, I have seen in tuberculosis, but very seldom, and I have also on one single occasion seen it in a tuberculin-negative patient. It seems not to have been reported in cases of sarcoid. Unilateral H. A. with both joint affections and E. nod. is seen in tuberculosis, in streptococcal diseases and others, but has not been reported to occur with Boeck's sarcoid. Acute rheumatism without H. A. or E. nod. is, as we know, the typical feature in rheumatic fever, but may, as stated, also be seen in tuberculosis, whereas it seems not to be found with sarcoid. The same applies to acute rheumatism with E. nodosum, but without H. A. Finally, E. nodosum without joint phenomena and H. A. may be seen both in tuberculosis and other conditions, but has not been reported in cases of sarcoid.

All this may perhaps seem extremely complicated. I have here been seeking to show that bilateral and unilateral hilar adenitis, E. nodosum and acute rheumatism seem to be related conditions and that none of them, whether isolated or combined, are characteristic for a particular etiology. Accordingly we cannot establish an etiological diagnosis on the basis of the clinical picture alone. The most valuable diagnostic aid is the tuberculin test, since a vesicular reaction speaks strongly for tuberculosis. But neither is this absolutely valid, and a positive Pirquet reaction in itself alone by no means implies a tuberculous etiology, as Vogt has rightly emphasized.

If we now regard both the hilar adenitis and the joint affections and erythema as allergic reactions to various noxae, the question then arises: What infection or infections can come into

consideration in this group of 20 cases of B. H. A., where tuberculosis and sarcoid can probably be excluded?

It is natural to think of *hemolytic streptococci*, in view of their known relation to rheumatic fever. Meanwhile Löfgren finds, firstly, that in his material B. H. A. is most frequently seen in the cases which are due neither to tuberculosis nor to *streptococci*. And, secondly, he has found the joint phenomena to be especially dominant in the group in which no signs of streptococcal infection could be discovered by aid of the antistreptolysin titer (AST), cutaneous reactions or cultures from the throat. Some of my cases can no doubt be supposed to have been due to streptococcal infection. In a man with B. H. A. and pronounced affections of the joints there were found hemolytic streptococci in pure culture from the throat, as well as vesicular cutireaction to streptococcal emulsion and an AST of 374. But Löfgren's investigations go to show that this is only a small part of the explanation.

It has been a matter of discussion whether what is called »true» rheumatic fever represents one of the causes of E. nodosum, with or without H. A. The question arises with equal justification as regards the B. H. A. The clinical picture may here be strongly suggestive of rheumatic polyarthrits, even though the cases are usually fairly mild and the characteristic sweating is absent. The age-distribution, with preponderance of women over 30 years old, is not concordant. Electrocardiographic changes are rather seldom noted, both in E. nodosum and B. H. A., and moreover they are also to be seen in case of tuberculous primary infection.

On the other hand, several of the patients have previously had rheumatic fever, some of them repeatedly, and the illness is sometimes initiated by angina. Edström (24) on follow-up examination of 48 patients with simultaneous acute rheumatism and E. nod. finds that 23 of them have got valvular disease of the heart, a finding which, moreover, stands in strong contrast to the observations of most other authors.

Löfgren finds no support for the assumption of a connection between true rheumatic fever and E. nodosum. As regards the B. H. A. I am also inclined to be doubtful as to the identity of the two conditions, in spite of the clinical resemblance. Now it is doubtful whether rheumatic fever represents a clearly delimited pathological unit in etiological respects. It may be that different

infections here come into play, and the whole matter then becomes to some degree a dispute about words.

Table 8.

Simultaneous Erythema Nodosum.

Tuberculosis	5 of 14
Boeck's sarcoid	1 » 10
Boeck's sarcoid?	3 » 6
Rheumatic type	16 » 20
Resp. aff.	0 » 3
Uncharacteristic	3 » 10
Hodgkin, leucemia, reticuloend. etc.	0 » 14
	<hr/> 27 of 77

From Table 8 it is seen that *E. nodosum* with B. H. A. is especially characteristic for the »rheumatic type», is less so for tuberculosis and is only seldom seen in case of sarcoid. Miliary condensations in the lungs have been recorded in 17 of the 77 cases. Table 9. If the patches are dense and persist for a considerable time the presence of sarcoid is very probable. In four cases of the »rheumatic type» the condensations were very inconspicuous. The judgment of such pictures is difficult. In some of the cases it may well have been a matter of stasis due to the pressure of the glands. The idea that rheumatic granulomas were present in the lungs seems rather far-fetched.

Table 9.

Simultaneous Signs of Miliary Spread.

Tuberculosis	2
Boeck's sarcoid	6
Boeck's sarcoid?	3
»Rheumatic type»	4
Uncharacteristic	2
	<hr/> 17

Both in the »uncharacteristic» cases and in the group which I have designated »rheumatic» I have in many instances observed that the tuberculin reaction, which during the highest stage of the illness had been weak or negative, in the later course of the disease or in the convalescent period became distinctly, and sometimes strongly, positive, a finding which might seem to point

towards a temporary suspension or reduction tuberculin sensitivity during the illness. In a couple of these patients and in one or two others among the tuberculin-negatives there was found, simultaneously with negative tuberculin reaction, undoubtedly calcified patches on the radiogram of the lungs, as sign of earlier tuberculous infection. These findings must presumably be said to speak against tuberculous etiology.

In the cases with tuberculous etiology perihilar condensations appearing together with the hilar adenitis seem to be a more prominent feature than in non-tuberculous cases, without being, however, so constantly present that it can be utilized for diagnostic purposes. Neither can the fact the hilar adenitis is far more pronounced on one side than on the other be utilized as an aid to diagnosis. The same applies to paratracheal glandular swellings which may not seldom be observed together with B. H. A.

Sometimes the hilar adenitis in patients with E. nodosum cannot be radiographically detected until several weeks after the erythema has appeared, a circumstance which may also be observed in cases of tuberculous E. nodosum attended by ordinary unilateral H. A.

It is extremely difficult to arrive at an exact diagnosis in cases of B. H. A. with the knowledge we at present possess. Chronic miliary tuberculosis may be characterized by tuberculin negativity, by negative findings as regards bacilli and by joint phenomena, and may be cured. We therefore cannot entirely exclude the possibility that within the group of »rheumatic type» and of uncharacteristic cases there may lie concealed some cases of tuberculosis.

On the other hand, Gravesen and others have shown that patients with B. H. A., joint pains and negative tuberculin reaction may present definite signs of Boeck's sarcoid several years later. The long observation period in most of my cases should here speak against such an eventuality.

The clinical differential diagnosis between Boeck's sarcoid and tuberculosis, or, if preferred, between ordinary tuberculosis and »non-caseating tuberculosis», may be very difficult. The radiographic picture of cystoid osteitis may be seen in both conditions. The plasma proteins give no guidance. Biopsy of the tonsils often fails to afford information. I wish specially to emphasize that, according to my own and other writer's experience (Björnstad (25), Bjerkelund, Löfgren, Opsahl), no weight whatever can

be assigned to the reaction to inoculation of B. C. G. or killed tubercle bacilli. The best aid to diagnosis is probably to be found in the tuberculin test and Kveim's reaction. Vesicular or very strong tuberculin reaction speaks strongly against sarcoid. Likewise negative Kveim reaction. A general idea of the importance of a positive Kveim reaction can doubtless not be attained until our knowledge respecting possible non-specific reactions has been extended. The question as to whether Boeck's sarcoid is called forth by tubercle bacilli I shall not enter into here.

Conclusion.

Bilateral hilar adenitis, with or without E. nodosum at the same time, occurring in cases where we find no material for biopsy or characteristic affections of other organs, will not quite infrequently be due to tuberculosis, especially where the tuberculin reaction is strongly positive, but also where it is weak or negative. Boeck's sarcoid will far more seldom be present and only where the tuberculin reactions are weak or negative. A large number of the cases are characterized by joint affections, and we must reckon with the existence of a separate group which together with unilateral hilar adenitis, E. nodosum, E. multiforme and polyarthritidis acuta may possibly bear relation to rheumatic fever.

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Thiouracil Treatment and Its Indications.¹

By

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When Astwood in 1943 introduced the use of thiouracil and its derivatives into clinical medicine, he added to the therapy of thyrotoxicosis a new method, which on account of the experience gained since is comparable with surgical and irradiation therapy.

The question of the indications is however still far from settled. While some authorities, such as Astwood (1) himself in United States and Meulengracht (2) in Denmark treat all or almost all cases of thyrotoxicosis with thiouracil, the majority of authorities in this field take a far more conservative standpoint (1).

At the meeting of the Finnish Association of Physicians (Finska Läkaresällskapet) in February 1947, Dr. Wijnbladh and Docent Frisk, invited guests from Sweden, spoke on this subject. In the discussion I (3) then gave my conception of the indications which for the present should be followed in Finland:

Thiouracil treatment in thyrotoxicosis is in this country indicated only:

1. As preoperative treatment in cases which do not react satisfactorily for iodine therapy.

2. As an independent method of treatment

- a) in cases of persistent or recurrent postoperative thyrotoxicosis, when a new operation is not indicated.

¹ Read before the Finnish Association of Physicians (Finska Läkaresällskapet), November 13th 1947.

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b) in the very few cases in which surgical treatment for some reason is counterindicated.

Since that time greater experience has been gained elsewhere as well as in this country and on that account it seems pertinent to revert to the question of thiouracil therapy and its indications. The subject is of current interest as our medical firms have brought methyl thiouracil into the market and the use of it seems to be on the increase. It seems that the indications have not always been correct and the results have, in some cases, been discouraging. It is motivated to return at first to the reasons for my comparatively conservative point of view in this matter.

It is not correct to use thiouracil in the preoperative treatment of thyrotoxicosis in all medium and severe cases as is largely done in the thyroid clinics in United States. The reaction to iodine in the majority of cases in this country quite satisfactory whereas in United States it is not so. Preoperative treatment with iodine takes only a fraction of the time required by preoperative treatment with thiouracil and can thus be carried out clinically, in a comparatively short time, at the same hospital where the operation is to take place, which is for several reasons particularly desirable. In those few cases in which the reaction for iodine is not considered sufficient to allow surgery without risk, treatment with thiouracil is resorted to. It should be carried out clinically, partly because these cases are too severe for outpatient treatment and partly for diminishing the risk for complications, in the first place aggranulocytosis. This refers also, of course, to cases of thyrotoxicosis in which surgical intervention is considered indicated, and in which the thyrotoxicosis either seems to be precipitated by iodine, or the disease has entered an unfavorable course during iodine treatment.

In connection with preoperative treatment with thiouracil there is reason to remind of the fact that the increased hyperemia does not cause technical difficulties during the operation if iodine is administered simultaneously with thiouracil for one or two weeks at the end of the treatment in the manner which has been customary in preoperative iodine therapy. An enlargement of the thyroid in connection with thiouracil medication does not occur if the dosage is moderate enough. I have not observed this occurrence in a single case. By bilateral resection or enucleation of adenomata the surgical treatment reduces the mass of hyperactive thyroid parenchyma

whereupon the tendency to spontaneous healing of the disease does prevail. The treatment with x-rays evidently reduces directly the secretory hyperactivity in the thyroid parenchyma. At the meeting of the American Association for the Study of Goiter in Chicago in July 1946 (1) I expressed, on the basis of the histological picture in the slides shown, the supposition that the influence of radioactive iodine («radio iodine») might be due to a diffuse proliferation of connective tissue which encloses the hyperactive follicle epithelium cells and thus prevents them from secreting their ineret into the circulation.

The surgical as well as the irradiation therapy in thyrotoxicosis is thus symptomatic and is directed against the pathologically augmented incretion from the thyroid and not against the cause of it, the «factor X», which is still unknown.

This refers to the treatment with thiouracil as well. It prevents the use of iodine supplied to the organisms for building up of active thyroid hormone. The histophysiological picture, characteristic of thyrotoxicosis, hyperactivity of the follicle epithelium with hormone forming secretion, incretion of thyroid hormone into the circulation, remains, but the hormone becomes inactivated «emasculated» on account of lack of iodine. The hypothyroidism thus ensued brings about an increase of the thyrotropic influence of the adenohypophysis, a fact which further accentuates the histophysiological picture just described.

These facts explain one of the greatest disadvantages of the thiouracil treatment, that it has to be continued for months, sometimes for years, before a lasting result can be obtained. This may be explained, as stated by Frisik (4), by the hyperactive follicular epithelium finally turning into involution. Another disadvantage, which is the greatest, is the toxicity of thiouracil and its derivatives, which manifests itself as a series of reactions of hypersensitivity, in the first place agranulocytosis. This concerns all thiouracil derivatives known at present, also methyl and propyl thiouracil, although they are less dangerous than thiouracil owing to their high effectivity and in consequence smaller dosage: They are also poisonous and in the first place with regard to the bone marrow. The risk of complications is reduced by close observation during clinical treatment, yet several examples show that it even then is to be reckoned with. Clinical treatment of a case of thyrotoxicosis with thiouracil until complete recovery is almost always impossible already for economical reasons. Ambulatory treat-

ment cannot, due to reasons mentioned above, be carried out without a considerable risk, if the patient is not resident at the same locality as the physician in charge and comes often enough for check-up. Furthermore, the patient must be acquainted with certain symptoms, in the first place such as sore throat, tenderness of the mouth, swollen lymphatic nodes on the neck and fever, which may denote agranulocytosis and which require immediate termination of medication, a visit to the laboratory for blood tests and to the physician in charge. This all presupposes, in addition to a minimum of intelligence on the part of the patient, a very good cooperation of the physician and the patient.

The facts just mentioned greatly reduce the indications for treatment of thyrotoxicosis with thiouracil and particularly with thiouracil alone, without operation. In addition comes the fact, that in Finland the waste majority of cases of thyrotoxicosis have a nodular goiter which, with all probability, will sooner or later indicate surgical treatment on account of mechanical pressure.

Among counterindications pregnancy should be mentioned as the fetus, as observed by Frisk (4), may be injured. Also intrathoracic goiter is a relative counterindication although, as mentioned earlier, the danger of the goiter increasing in size is minimal if the dosage is moderate.

It seems evident that in Finland we still have reason to take a comparatively conservative attitude to the treatment with thiouracil and to limit the indications mainly to those just mentioned. It is however no doubt tempting to treat ambulatorily with thiouracil certain slight cases of thyrotoxicosis without a goiter or with a small goiter, which is either diffuse or contains small, quite soft nodules, and this may even be considered as justified if the conditions previously mentioned are fulfilled. The danger involved should however always be kept in mind and the matter be dealt with accordingly. I will not refrain from mentioning that I have myself treated six cases of thyrotoxicosis ambulatorily with thiouracil, one case without and five cases following previous clinical treatment. One of the later cases had clinically been treated with x-rays and four with x-rays and thiouracil simultaneously. In all six cases the final result was favourable. The treatment was discontinued, however, in two of these cases on account of edema of the eyelids without signs of hypothyrosis and in one case due to swollen lymphatic nodes on the neck and eczema. In a fourth case methyl thiouracil was exchanged for propyl

thiouracil when edema appeared on the ankles, which however disappeared when exchanging the preparation. Thus of six ambulatorily treated cases only two did not get complications.

As I mentioned earlier in a discussion at the meeting of the Finnish Association of Physicians in February 1947 (3), I have endeavoured to find a solution to the problem of treatment with thiouracil in slight, suitable cases of thyrotoxicosis by using x-rays and thiouracil simultaneously. The purpose of this procedure is to reduce at the same time the reaction to x-rays by thiouracil and to shorten the treatment with thiouracil by means of x-rays. In this manner two qualitatively completely different, inhibiting influences will be applied at the same time, a procedure, which according to experience, may be advantageous to the outcome.

The results may be considered encouraging, with the reservation, which the short time of observation — not more than one year — accounts for. The procedure has gradually developed into the normal method in cases in which an attempt with thiouracil therapy, not to be followed by operation, may be considered as indicated in accordance with the principles stated previously. The patients have as a rule been hospitalized for one month. The treatment has been started with thiouracil and a few days later, when the symptoms of thyrotoxicosis have subsided, x-ray therapy has been initiated. It has been administered by Docent Jansson. Both lobes of the thyroid have first been irradiated with 100 r. and later three times with 150 r., altogether eight irradiations of 1,100 r. Daily treatments were given. Reactions which might have caused postponement of the x-ray treatment were not observed in any of these cases. The therapy with thiouracil was continued, altogether for about four weeks. During the last week the patients were allowed to be up for a daily increasing time and during the last days also out of doors. In the beginning the thiouracil medication was continued ambulatorily after the discharge from the hospital, but since it in all these cases had to be stopped due to complications, it has been discontinued at the discharge from the hospital. The patients have been called for a check-up two to three months later and, if required, another series of x-rays has been given clinically or ambulatorily, with or without simultaneous thiouracil treatment, owing to the conditions.

This series of thyrotoxicoses treated with thiouracil and x-rays comprises thirteen cases. Three of them are relapses and three persistent postoperative cases and seven are cases of slight thyro-

toxicosis in which surgery was not considered indicated. In five of these thirteen cases one or two additional series of x-rays were given: In one case two and in two cases one series ambulatorily without thiouracil, in one case one series with thiouracil clinically and in one ambulatorily. Five patients have been considered free from symptoms and six distinctly improved; two cases are still at the hospital at the end of their treatment and free from symptoms of thyrotoxicosis. One patient was later operated on. In this case treatment with x-rays and thiouracil was resorted to as the consulted surgeon first considered advanced age, intrathoracic goiter, sclerosis of the aorta, myocardiac failure and severe coronary insufficiency counterindicate operation. The patient improved considerably and was two and a half month later operated on after routine preoperative iodine treatment. She improved further although her working capacity is somewhat reduced due to stenocardia. Four of the patients in this series had symptoms of coronary insufficiency, which improved considerably after the treatment.

It seems, as stated above, as the results of my trial with clinical treatment of thyrotoxicosis with thiouracil and x-rays simultaneously were encouraging and I intend to continue it.

Among my cases only treated with thiouracil one should be mentioned, which was treated ambulatorily partly because the symptoms were so slight that the patient was able to be at work and partly because the contact between patient and physician was considered reliable. The treatment which was carried on for a period of six and a half months was continued by Docent Frisk when the patient was visiting Sweden. The result was a complete absence of symptoms and is still so about six months later.

Another case also deserves mentioning. A woman, forty-three years of age, with a myocardial affection and heart failure without goiter or thyrotoxicosis, did not any more get along with her usual dosis of 0.1 grams of digitalis. She received propyl thiouracil 0.12 grams daily during one month clinically together with x-rays in the manner described, with a view to reducing the thyroid activity so much as to regain compensation. She became actually completely compensated with her previous dose of digitalis but the time of observation is still too short to allow the final result to be definitely evaluated.

My material now includes twenty-four cases treated with

THIOURACIL TREATMENT AND ITS INDICATIONS.

methyl¹ or with propyl¹ thiouracil, methyl thiouracil in doses of 0.1—0.4 grams and propyl thiouracil in doses of 0.1—0.2 grams daily. The dosage was thus very moderate and the indications have been comparatively rigorous.

Three circumstances have principally been the cause of the question of thiouracil treatment and its indications having been taken up for discussion again although my own material is still so small. The first two ones have already been mentioned: Finnish medical firms have brought methyl thiouracil into the market, and in treatment with thiouracil and x-rays simultaneously I consider a way may have been found to a decrease in the risk involved as well as for an increase of the effectivity of thiouracil therapy. The third reason is the high incidence of complications in my series.

Untoward symptoms, to be considered as complications, occurred in a total of nine in my series of twenty-four cases, including slight rises of temperature, viz. one case of hemorrhagic diathesis with reduced prothrombin index and prolonged coagulation time, one of edema of the ankles, one of lymphomata on the neck and eczema, one of granulocytopenia, one of very acute, severe agranulocytosis during clinical treatment with methyl thiouracil and x-rays, two of swollen eye lids without symptoms of hypothyrosis and two of exophthalmos at the end of the treatment. At the meeting of the Finnish Association of Surgeons a fortnight ago BISTRÖM (5) described a case treated ambulatorily with methyl thiouracil; the patient developed myxedema and goiter during an interval of eighteen days between two check-ups. This case shows only the importance of close observation; it does not concern hypersensitivity to thiouracil.

I still consider it questionable whether the high incidence of complications in my small series, despite moderate dosage and rigorous indications, is accidental or whether an increased sensitivity to thiouracil is actually prevalent in Finland, possibly in connection with post-war nutritional conditions. The increased tendency to spontaneous leucopenia might speak for this being the case.

My statements related here indicate, in my opinion, that a certain cautiousness in treatment with thiouracil in Finland is advisable.

¹ Propyl thiouracil (Lederle Laboratories, New York, U. S. A. and Pharmacia, Stockholm, Sweden), Methyl thiouracil (Astra, Södertälje, Sweden). The preparations have kindly been placed at my disposal by these firms.

Summary.

In the therapy of thyrotoxicosis the treatment with thiouracil is important enough to be compared with surgery and irradiation.

The question of the indications is still open and opinions among workers in this field vary considerably. Local conditions in different parts of the world should be taken into consideration in this respect.

In Finland the reaction to iodine is excellent and preoperative treatment with thiouracil is comparatively rarely indicated.

The majority of cases of thyrotoxicosis in Finland are accompanied by a nodular goiter which, with all probability will sooner or later indicate surgical treatment due to mechanical pressure. On that account treatment with thiouracil alone, without subsequent operation, is comparatively rarely indicated.

In the authors series of twenty-four cases treatment with propyl and methyl thiouracil in moderate doses (propyl thiouracil 0.1—0.2 and methyl thiouracil 0.1—0.4 grams daily) and rigorous indications as well as close observation complications occurred in nine cases, one of them granulocytopenia and another a fulminant agranulocytosis. The author considers it questionable whether this is an accidental occurrence or whether the deficient food conditions and the increased tendency to spontaneous leucopenia prevailing in Finland may possibly be factors of bearing in this subject.

The indications for treatment of thyrotoxicosis with thiouracil derivatives in Finland are according to the author's conception principally as follows:

1. As preoperative treatment in cases which do not react satisfactorily for iodine therapy.

2. As an independent method of treatment

- a) in cases of persistent or recurrent postoperative thyrotoxicosis, when a new operation is not indicated,

- b) in the very few cases in which surgical treatment for some reason is counterindicated.

Furthermore treatment with thiouracil may be considered indicated in slight cases of thyrotoxicosis with none or a small goiter which is completely diffuse or contains small, quite soft adenomata. Ambulatory treatment with thiouracil always involves a considerable danger and it may be justified only when

THIOURACIL TREATMENT AND ITS INDICATIONS.

supervision is as effective as possible, which implies a perfect collaboration between physician and patient. The author has endeavoured to find a solution to the problem of decreasing the risk involved in treatment of thyrotoxicosis with thiouracil and at the same time to increase the effectiveness of the therapy by clinical treatment for one month to three months and x-rays simultaneously. After an interval of two to three months, the treatment may be completed by an additional series of x-rays, clinically or ambulatorily as may be required, with or without simultaneous treatment with thiouracil. Thirteen cases have so far been treated and the results obtained seem to be encouraging. A certain cautiousness in treatment of thyrotoxicosis with thiouracil in Finland is, according to the author's viewpoint, advisable.

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Myelomatosis.

Examination of Clinical Material.

By

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Introduction.

Myelomatosis is a disease which has during several decades been a subject of special interest for clinicians, histologists and biochemists. In view of the voluminous literature that has been published respecting the disease it might hardly be expected that new case reports would throw further light upon its pathogenesis and its clinical aspects. — The improved methods adopted in recent years for qualitative and quantitative determination of the serum protein fractions have brought this disease again into the limelight. In the Medical Department B of the Rikshospital systematic investigations respecting the behaviour of the serum protein in cases of myelomatosis have been pursued for a considerable time, and the material in the department has now been gone through in order to see whether the protein changes found accord with the investigations reported in recent years from American quarters. It shall, however, at once be remarked that in our department there was no opportunity of determining the serum protein fractions by electrophoresis and ultracentrifuging. The other main question that shall be taken up for consideration is the frequency and degree of the renal lesions in myelomatosis.

Most authors are of the opinion that the origin of the myeloma is a tumorous proliferation of the plasma cells of the bone marrow.

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MYELOMATOSIS.

Waldenström (1944) describes a disease which he designated incipient myelomatosis or «essential» hyperglobulinemia, and he discusses in this connection the possibility that the primary element in the nature of myelomatosis is an abnormality in protein metabolism with secondary deposition in the bone marrow. The serum protein changes recorded by Waldenström in the published case were probably in some instances indicative of a particular fundamental disease, and his assumption of a disturbance in protein metabolism as being the primary element in myelomatosis has not remained unchallenged. Both in the elder and in the newer literature four types of myelomas are mentioned: the myeloblast type, the lymphoblast type, the erythroblast type and the plasma cell type. The clinical picture is identical in these four types. It is only their histology that distinguishes the different tumours. On the basis of exact cytological studies of bone marrow biopsies, Gormsen (1942) concludes that there are two cell types which are of interest in myelomatosis, namely, plasma and reticulum cells. The atypical plasma and reticulum cells may together be regarded as myeloma cells. Micrometry of the myeloma cells showed that they are in general larger than the normal plasma cells, and further that there is a disarrangement of the nucleus/plasma relation in favour of the nucleus. The nucleoli of the myeloma cells are larger than normal, mitoses are relatively seldom seen and the mitochondrial structure is different from that seen in the normal plasma cells. In order further to distinguish between normal and pathological plasma cells we have special staining methods, of which Pappenheim's methyl green pyronin is the most suitable. When speaking of the morphology of the cells, the relation between leucemia, and then especially the acute plasma cell leucemia, and myelomatosis must also be mentioned. Gormsen (1942) adopts no definite standpoint to the question whether plasma cell leucemia is a disease *sui generis* or whether myelomatosis in its course may become leucemic. Meyer and coworkers (1945) describe a case of acute plasma cell leucemia with several types of plasma cells in the peripheral blood. Many of these cells were immature, and the authors think that there here exists an acute plasma cell leucemia with serum protein changes of the same kind as are found in myelomatosis. If it is true that the cells present in plasma cell leucemia are normal reticulum cells which resemble plasma cells (Whitby and Britton, 1946), the differentiation between these cells and myeloma cells should not involve too

great difficulties (Rubinstein, 1945). It is chiefly in the terminal phase of myelomatosis that myeloma cells are found in the peripheral blood, and they are most frequently seen in the diffuse type of myelomatosis. The clinical course of this form of myelomatosis is leucemic, with plasma cell infiltration in the bone marrow and in several organs. This gives a natural explanation of the fact that several authors have spoken of transition of myelomatosis to leucemia.

Plasma cell proliferation may result in formation of solitary nodules (plasmocytoma, solitary myelomas) or a more diffuse affection of the bone marrow (myelomatosis). The myelomatosis may show two forms of development, namely, multiple myelomas or, more rarely, diffuse myelomatosis. Solitary myelomas occur chiefly in youth, and histologically they are characterized by the fact that the cells are more mature than in case of myelomatosis. The growth of the plasmocytomas is expansive, sometimes infiltrating. Usually they take a benign course and good results have been attained by operative removal of the tumour. But the plasmocytomas can also be malignant, and a fatal issue in less than a year has been reported. In order to diagnose solitary myelomas with certainty the time of observation must be at least 4 years, and even after an observation period of 8 or 10 years the disease has sometimes proved to be myelomatosis. Diffuse myelomatosis is very rare. Ask-Upmark (1945) has collected 25 cases from the literature and mentions in addition one case of his own. Some of these cases, however, are doubtful. Multiple myelomas represent the most frequent form of plasma cell proliferation in the bone marrow, and these are multilocular in their structure and have not arisen through metastasis from a solitary nodule, as Magnus-Levy (1932) assumes. Tybjerg-Hansen (1943) maintains that the diffuse and the nodular forms may represent different stages of myelomatosis.

The symptomatology varies greatly. Geschickter and Copeland (1936) mention 6 cardinal symptoms that attend multiple myelomas, and two or more of these are usually present at the same time. The symptoms are the following: Multiple affections of the skeletal system in adults, pathological costal fractures, Bence-Jones (B-J) proteinuria, characteristic pains in the back with early-occurring paraplegia, an otherwise inexplicable anemia and, finally, chronic nephritis with N-retention, low blood pressure and increase in serum proteins. Meanwhile, cases have been de-

scribed which presented none of these characteristics, while other symptoms have been more prominent, such as hyperproteinemia with inversion of the A/G ratio, hypercalcemia with normal or high serum phosphorus content, evidence of autohemagglutination in the counting chamber, Jersild's anticomplementary reaction and reduced content of citric acid in the blood (Ask-Upmark). None of the symptoms in this latter group are pathognomonic. Of less commonly seen symptoms may be mentioned: affections of the joints in the form of synovitis and periarthritis (Mägnus-Levy, 1932), exanthema of which the nature has not been made clear owing to lack of histological examinations, zoster resulting from vertebral destruction, as well as diabetes insipidus occasioned by infiltration of myelomatous tissue in the hypophysis (Jansson, 1946).

No systematic investigations respecting the serum proteins in case of solitary myelomas are reported in the literature. On the other hand, we find many searching investigations regarding myelomatosis. For this purpose various methods have been employed, namely, desalination, electrophoresis and ultracentrifuging. Mägnus-Levy, Kellhæk, as well as Bondorf and coworkers and several others regard the hyperproteinemia as being a consequence of increased euglobulin content, while Gutman and associates (1941) in 38 cases of myeloma found either an increase of pseudoglobulin I + euglobulin, or else only an increase of pseudoglobulin I, and as a third alternative an increase of pseudoglobulin II. On the basis of electrophoretic studies the serum proteins are divided into the following globulin fractions: α_1 and α_2 , β_1 and β_2 , and γ . After exhaustive studies of the serum proteins in myelomatosis Kellwick (1940) came to the result that the sera either showed the normal number of protein components or, in addition to these components, a globulin fraction not normally observable. Kellwick showed that in the great majority of sera from myeloma patients there is an increase in γ globulin, and that the deviation from the normal content of γ globulin increases in every case with the total protein content in the serum. The same author further found in one case a rise in globulin β_2 . Longsworth and coworkers (1939) and Olhagen (1947) have also reported cases with increase of β globulin. In myeloma sera there have further been found spontaneously crystallizing proteins (Bondorf and associates, 1938, Packalen, 1939). Several authors have raised the question whether the hyperproteinemia in myelomatosis is a consequence of in-

creased content of the normal protein components in the serum, or whether there are other protein compounds which through a coincidence happen to behave in the same manner as regards precipitation. Shapiro and associates (1943) believe that the sera may contain hitherto unclassified proteins. From the results of electrophoretic studies of albumin Kekwick concludes that the components present in excess in myelomatosis can be distinguished qualitatively from the corresponding components in normal serum.

Moore and coworkers (1943) divide the myeloma cases into three main groups on the basis of the changes in the serum protein. To the first group are assigned cases with hyperglobulinemia due to increased content of γ globulin. The second group comprises cases with an unusual type of proteins — Bence-Jones proteins, and as forming a third group are reckoned cases with apparently normal serum proteins. In the older literature we find mention of cases with considerable quantities of B-J proteins (cit. Fellmer and Fowler), but by use of improved methods it has been proved that the concentration of B-J protein in serum usually lies between 0.2 and 0.5 per cent (Apitz, 1940, Moore and coworkers, 1943). The serum albumin is normal or slightly reduced, the fibrinogen concentration normal or slightly increased. Changes in the colloid-osmotic pressure are seldom found, since the increase that may occur in the serum proteins is due to macromolecular proteins with relatively slight colloid-osmotic activity.

The pathogenesis of the renal lesion in myelomas is still in dispute. It is a fact that normal glomeruli are passable for B-J proteins. Mainzer (1932) believed that the iso-electric point of the proteins was determinative for their filtrability through the glomeruli. According to that author the iso-electric point of the B-J proteins should lie between 6.6 and 7.0, and all protein with pH above 6.0—6.6 would be able to pass through a normal renal filter. By electrometric titration Jervell and Nicolaysen (1932) demonstrated that the isoelectric point for B-J proteins was at pH 4.0—4.25. It is the size and structure of the molecules that determines whether they can pass through intact glomeruli. Several authors (including Bell, 1933) have shown that excretion of B-J proteins may proceed during a considerable time without entailing damage to the capillaries of the glomeruli. On the other hand, excretion of albumin alone through the kidney is an indication of glomerular damage, probably due to anoxemia (Bell). The filtered B-J proteins will sometimes be reabsorbed in the tubuli cells (the

arthrocytosis phenomenon) and sometimes there will come an occlusion of the tubuli as a result of cylinder formation. Bell regards the formation of cylinders as the chief cause of renal insufficiency in cases of myelomatosis. Atrophy and dilatation of the renal canals are not indicative of primary degeneration, but of secondary atrophy. Ehrich (1932) advanced this mechanical explanation of the renal damage before it was suggested by Bell. The tubular changes then lead to fibrosis, with development of contracted kidney. Contrary to the views here advanced, Randerath concludes that the pathogenesis is of the same nature as for depot nephrosis and that the development of nephrohydrosis is a very late symptom. This conception is supported by Apitz (1940), who in a series of investigations has shown that purely nephrotic renal changes occur in cases where a mechanical occlusion of the tubules can be excluded. Perla and Hutner (1930) believe that renal lesion has no relation to the myelomatosis *per se*, and they point to the frequent occurrence of arteriosclerotic diseases of the kidneys in the age groups in which myelomatosis is most often found. According to Apitz arteriosclerotic changes in renal vessels are not more common or more pronounced in myeloma kidneys than in control material. The renal changes in myelomatosis have also been studied by Lagercrantz (1944), who found that the glomeruli and vessels are in general undamaged. The picture is characterized primarily by lesion of the tubular epithelium and secondarily by formation of cylinders. The question whether in the later stages there develops a purely nephrotic or a hydronephrotic contracted kidney is of little or no practical importance.

Author's Material.

In 1933 the first case of myelomatosis was clinically diagnosed in Med. Dept. B of the Rikshospital. Since then, altogether 17 cases of myelomatosis have been treated in the period 1933—1946. The incidence of the cases in the different years is shown in Fig. I. More than half of the myeloma cases were diagnosed in the last three years. This accumulation of myeloma cases in these years cannot be taken as indicative of increased frequency of the disease, and neither can it probably be ascribed to more accurate diagnosis, as the staff of the department has all the time been »myelomatosis-minded». One is therefore most inclined to explain the increase in frequency as coincidence. The distribution according

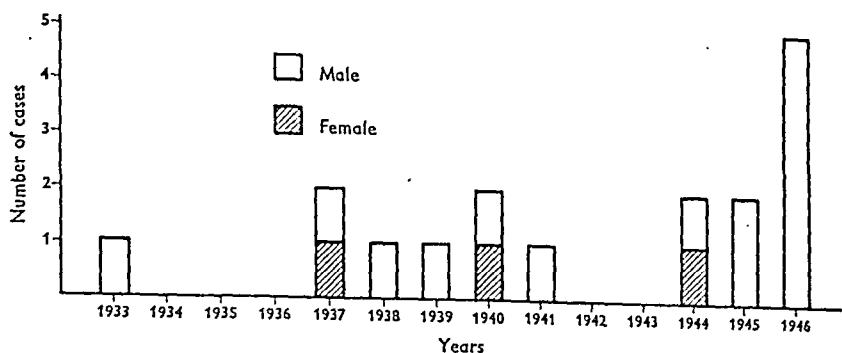


Fig. 1. Cases of myelomatosis in the years 1933-47.

to sex, and to age at the time the disease was diagnosed, will be seen from Table I. In concordance with earlier publications there is found a considerable preponderance of man (about 5 : 1) and most of the cases occur after the age of 50 years (13 out of 17 cases).

Table I.

Sex and age distribution in myelomatosis.

Sex	A g e					Total
	36-40 yrs.	41-50 yrs.	51-60 yrs.	61-70 yrs.	71-80 yrs.	
Female	1	1		1	2	3
Male	1	1	7	3	2	14
						17

In three cases the diagnosis for admission to the department was correct. Two of these patients were transferred from other medical departments where the disease had been diagnosed. Apart from these last-mentioned patients, we have consequently only one case in which myelomatosis was diagnosed before admission to hospital (this patient was admitted by the Chief of Med. Dept. B). The diagnoses for admission were the following: Chronic kidney disease, 8 cases. Purpura, 1 case, Melæna, 1 case, Leucemia, 1 case. Tumor orbitae, 2 cases, Spondylitis (?), 1 case, And, finally, 2 patients admitted for observation. These symptomatic diagnoses give us at once an idea of the polymorphic symptomatology of myelomatosis. On going through the case histories it is seen that pains in back and extremities represented the predominating symptom in 12 cases. Typical symptoms of anemia were found in

6 patients and recurrent bleeding from the skin in one case. Of the two patients who consulted a doctor on account of protrusio bulbi one had previously had pains in the back. In two cases the case histories were uncharacteristic.

In all the patients, except one, examination of the blood revealed hypochromic anemia with hemoglobin values between 80 and 31 per cent. Enumeration of white blood corpuscles and differential counts showed normal figures. Thrombocytes were counted in 10 cases, in only one of which thrombocytopenia was observed. Both the patient with purpura and the melæna patient had a normal number of blood plates. The tendency to bleeding in these cases must probably be ascribed to vascular changes.

The findings respecting the urine are set forth in Table II. It appears from this Table that all the patients had proteinuria on admission, and the proteinuria persisted during the whole time they were in hospital. Further were found B-J proteins (investigated by boiling test and the 10 % HCl test) in all the patients, except one. The renal functions were examined by urea clearance tests and by dilution and/or concentration tests in the urine. The results are given in Table II. At the first examination the urea clearance was normal in 4 patients, while in the others it was more or less reduced. The dilution and/or concentration test showed normal figures in 3 of the 16 cases investigated. On admission the blood pressure was normal in all except two cases, and clinically observable edema was found in 2 patients.

The analyses of serum protein were made *ad modum* Howe. The results are shown in Table III. The total proteins showed a slight rise at the first examination in three patients and a considerable rise in seven patients. Normal value was found in four cases and moderate hypoproteinemia in three. The serum albumin showed different degrees of reduction in 12 patients at the first examination, in the remainder normal figures. The globulin content was in no case hyponormal at the time of admission to the hospital. In 11 cases it was increased, sometimes considerably, the highest value being 13.38 per cent. In two patients the A/G quotient lay at about the upper normal limit, in four patients it lay around the lower normal limit and in the remainder the quotient was more or less inverted, with 0.05 as the lowest value. The Takata-Ara test and the formol-gel test were not systematically carried out in the material, and therefore the results are not entered in the table. In the cases where these tests were made

Table II.

Renal function tests and urine findings in myelomatosis

Case No.	Date	Urea in plasma, mg %	Urea clearance in % of the normal	Urine			Edema	Blood pressure on admission
				Dilution and/or concentration test	Proteinuria	Bence-Jones proteinuria		
1	22/11 1934	89	19	1012—1019	+	+	+	110/80
	19/12 "	94	19.5					
2	9/1 1935	182		1004—1029	+	+	—	130/75
	3/2 1937	31	92					
	14/4 "	28	94					
	20/12 "	35	120					
	2/3 1938	35	84					
	21/10 "	29	84					
3	17/3 1937	332	7	1010—1014	+	+	—	135/85
	21/3 "	331	5					
4	20/1 1938	42	83	1016—1023	+	+	—	120/70
	16/5 "	25	85					
	23/9 "	35	117					
5	31/10 1939	50	46	1014—1022	+	+	—	130/70
	19/9 1940	38	43					
6	2/10 "	42	27	1010—1014	+	+	—	135/85
	17/5 1940	80	13					
7	16/10 1940	37	35	1026—1028	+	+	—	140/90
	21/2 1941	32	49					
8	8/8 1941	101	22	1012—1014	+	+	—	120/70
	27/8 "	111	16					
9	12/4 1944	128	16	1010—1012	+	+	—	120/70
	3/7 "	210	10					
	11/7 "	287	11					
	23/7 "	313	5					
	9/8 "	267	7					
	11/11 1944	31	45					
10	11/11 1944	31	45	1028—1029	+	+	—	110/75
	19/3 1945	59	117					
11	2/5 "	35	63	1019—1020	+	+	—	140/85
	21/9 1945	53	42					
12	22/10 "	51	65	1022—1023	+	+	+	140/70
	30/10 "	63	64					
	16/1 1946	30	73					
13	22/3 "	47	37	1012—1012	+	+	—	170/100
	23/8 1946	97	44					
14	23/8 1946	97	44	1013—1013	+	—	—	120/90
	16/10 1946	104	20					
15	2/11 "	114	7.4	1010—1012	+	+	—	140/70
	16/10 1946	52	68					
16	2/11 "	52	68	1018—1019	+	+	—	120/60
	16/10 1946	47	52					
17	22/1 1947	47	52	Not perf.	+	+	—	170/110
	19/10 1946	166	11					

there was seen complete concordance between the results thereof and the changes noted in the serum protein. The same is seen to be the case as regards the sedimentation reaction, as there is regularly found a considerable rise in the S. R. with low A/G quotient. The calcium and phosphorus content in serum was de-

Table III.

Serum proteins, calcium and phosphor contents in blood at myelomatosis.

Case No.	Name and journal No.	Date	Serum proteins					Sedimentation rate	Ca mg %	P mg %	Phosphatase E.	Remarks
			Total %	Alb. %	Glob. %	Rest-N mg %	A/G					
1	J. B. 3234/33	30/11 1933	6.16	3.21	2.35	42.1	1.09	59	13.7	4.44	4.3	
2	N. T. 5794/37	2/2 1937	7.61	4.99	2.08	27.8	2.4	30	11.0	4.1	2.6	
		12/3 »	8.65	5.95	2.7	26.00	2.2					
		16/7 »	7.85	4.45	2.77	25.6	1.6					
		9/9 »	7.36	4.55	2.81	32.55	1.62	59				
		2/1 1938	6.47	3.9	2.57	31.68	1.52	97				
3	D. V. 7634/37	17/3 1937	8.17	5.16	2.32	184.00	2.22	54	9.3	16.8	1.4	
4	K. B. 2587/38	20/1 1938	7.75	4.19	3.26	36.02	1.38	80	11.4	5.4	3.4	
		23/9 »	5.72	2.99	2.73	41.23	1.1	85				
5	H. W. 1910/39	30/10 1939	8.22	3.43	4.79	28.6	0.72	80	10.3	6.3	2.6	
		4/9 1940	9.42	2.94	6.48	25.2	0.46	92				
6	L. B. 9880/40	18/5 1940	14.6	2.15	12.45	76.8	0.17	160	11.7	5.6	1.5	
7	E. S. 3262/40	9/8 1940	10.72	2.88	7.84	23.4	0.37	145	10.2	3.9	6.7	Aker Hospital
		17/10 »	11.02	2.84	8.18	30.4	0.35	130	10.9	5.1	5.7	
		11/1 1941	11.56	2.74	8.76	22.1	0.31	135				
8	J. J. 963/41	1/8 1941	5.68	3.5	2.18	78.5	1.6	12	11.6	10.5		
		15/9 »	6.01	4.12	1.54	69.8	2.9	18				
9	O. M. 12808/44	3/6 1944	8.65	2.53	6.12	57.3	0.41	143	12.8	5.3	5.2	Drammen Hospital
		1/9 »	7.17	2.18	4.99	156.00	0.44	150			4.2	
10	R. J. 5014/44	9/4 1944	7.2	4.2	3.00		1.4	100	9.0	3.5		Ullevål Hospital
		12/11 »	6.98	3.12	3.86	31.00	0.8	80	9.4	4.7	1.9	
11	E. G. 9229/45	17/3 1945	13.61	2.42	11.19	49.00	0.22	140	11.9	5.9	1.8	
		21/8 »	11.78	2.63	9.5	50.8	0.28	130	11.4	5.4		
12	O. H. 3291/45	27/9 1945	7.56	4.32	3.24	27.3	1.33	80	9.6	4.0	3.1	
		9/10 »	6.51	4.37	2.14	28.2	2.04	57				
13	O. G. 1416/46	11/1 1946	9.29	2.52	6.77	44.3	0.36	130	9.5	3.2	1.1	
		22/1 »	9.35	2.27	7.08	19.1	0.32	124				
		23/8 »	9.15	2.79	6.36	27.6	0.43	120				
14	I. F. 2073/46	25/8 »	15.5	2.12	13.38	135.00	0.15	160	9.6	5.3	2.2	
		1/10 »	15.32	2.28	13.04	155.00	0.17	160	9.7	5.7	2.4	
15	O. N. 4139/46	16/10 »	5.64	3.3	2.34	56.5	1.4	38				
		22/10 »	6.77	4.06	2.71	44.3	1.5					
16	M. G. 4156/46	17/10 »	10.76	2.24	8.52	27.4	0.26	160				
		15/11 »	11.55	2.46	9.09	30.2	0.27	155				
		16/1 1947	11.79	0.59	11.2	30.38	0.05	161	9.6	3.9	2.3	
17	O. S. 4258/46	17/10 1946	12.14	1.59	10.55	118.1	0.15	149				

terminated in 15 patients (Table III), 8 of whom had normal serum calcium, while in the others the content lay around the upper normal limit, and in one case it showed a considerable increase. The phosphorus content was increased in two patients, and the content of phosphatase in the blood was found to be normal in all cases.

Sixteen patients were radiographed. In 14 cases there were found multiple clear spaces in the skeletal system, such as are seen in myelomatosis. In all cases there was found considerable osteoporosis, and in two patients this was the only radiographic finding. Even though there are here found no skeletal destructions on systematic photography of the skeletal system, that fact does not justify the diagnosis of diffuse myelomatosis. These two patients have not been autopsied, so that the question whether there here really existed a diffuse form of the disease cannot be answered. In the literature are mentioned several cases in which the clinical course suggested diffuse myelomatosis, while post-mortem examination revealed myeloma nodules in the bone marrow. In view of this fact we find it most correct to catalogue all our cases as *multiple myelomas*. Multiple fractures were present in 7 cases and radiographic signs of pleural affection in 5 patients. The frequent occurrence of pleural affection is due to the myelomatous changes in the ribs having extended directly to the pleura. Tybjerg-Hansen has described a case of peribronchial infiltration by myeloma cells where the radiographic diagnosis was verified at autopsy. One of our patients presented a similar radiogram of the lungs, but we have not had an opportunity of verifying our probability diagnosis by post-mortem examination.

Sternal puncture was performed on 14 patients, in all of whom there were found changes typical of myelomatosis. The great diagnostic value of biopsies from the bone marrow in cases of myelomatosis is strongly emphasized by Beizer and coworkers (1942). These authors found that among 10 myeloma patients one had normal bone marrow, while the others showed typical changes.

All the patients were dead at the time of going through the material. Estimation of the duration of life for myelomatosis patients can be only approximative, since it is difficult, owing to the often uncharacteristic initial symptoms, to fix time at which the disease began. The probable duration of life in cases of myelomatosis is reckoned at about $2\frac{1}{2}$ years, but with widely varying extreme limits (from 3 weeks to 15 years). In the present material the time of survival ranged from 1 to $3\frac{1}{2}$ years, the average being 1.8 years. In the great majority of cases the cause of death was cachexia (13 cases). Two patients died of pneumonia and two in uremic state. Seven of the patients died in the hospital, and in these cases post-mortem examinations were made. From the records it appears

that in all cases the autopsy revealed plasma-cellular infiltrates in the osseous system, *i. e.*, changes typical of myelomatosis.

As mentioned in the introduction, the character and degree of the renal changes represent one of the main problems in this publication. There was unfortunately no opportunity of re-examining the histological preparations from the kidneys, so that the data here submitted come from the autopsy records. It is therefore most correct to present the results purely in summary form. In the renal changes noted those occurring in the tubules predominate. In all cases there were found degenerative changes of the tubular cells. In 4 cases these changes were of moderate degree, and there was here no demonstrable occlusion of the lumen. In the remaining three cases were found casts from the lumen of the tubules, atrophy and dilatation of the renal canals, as well as increase of the interstitial connective tissue. Glomerular changes were noted in 4 patients. The glomeruli showed capsular thickening and in three cases hyalinisation. Arteriosclerotic changes of arterioles and vasa afferentia were found in the preparations from four autopsies. Further, there was in one case noted amyloid substance and in one case marked deposition of calcium in the kidneys.

Discussion.

In Norwegian medical literature we find case-reports concerning myelomatosis (Harbitz, 1903, Tschudi-Madsen, 1918, Jervell, 1932, Salvesen, 1938), as well as Bøe's investigation of clinical material comprising 10 cases (1945). In the present material both serum proteins and renal function have been systematically investigated — and in case of some patients during a long period, whereby a solid basis for discussion should be obtained.

Before proceeding to discuss the changes in serum protein we must speak of the normal values. As already mentioned, the serum protein content was determined *ad modum* Howe by the same methods as were employed by Salvesen (1926) for determination of normal values. Bing and coworkers (1946) have adopted Henriques and Klausen's method, which is said to give somewhat more exact results than Howe's method. These authors showed that the normal limit for the globulin content lies somewhat higher after the age of 35 years, and their figures for the normal

values of the proteins differ somewhat from Salvesen's. Lange (1946) puts the limits for total protein at 6.3—8.86 per cent (average 7.45 per cent). Salvesen's normal values are as follows (the figures for males and females are fairly equal, and the values here given are for males): Total protein 6.53—7.96 per cent (average 7 per cent), albumin 3.95—5.24 per cent (average 4.44 per cent) and globulin 1.96—3.16 per cent (average 2.58 per cent). The A/G quotient is stated to be 1.43—2.26 (average 1.72). If we keep strictly to these figures we see that at the first examination only Case 10 (Table III) had normal total protein and normal distribution of the protein fractions. In Cases 2, 3, 4 (at first examination), 12, and possibly Cases 1, 8 and 15, the total protein and the A/G quotient lie so close up to the normal that it must be deemed most correct to reckon also these cases among those having normal serum proteins. Consequently, in the present material 8 out of 17 patients are found to have normal serum protein content with the technique employed in the analysis. In the remaining 9 cases there was noted hyperproteinemia with hyperglobulinemia (highest value 13.38 per cent, in Case 14) and inversion of A/G. The lowest value for A/G, 0.05, was found in Case 16. *Over half of the patients had hyperglobulinemia, low A/G quotient and increased content of total protein in serum.* In previously published works (cited by Gormsen) the frequency of hyperglobulinemia is reported to be from 68 to 86 per cent, and Bayrd and Heck (1947) found total protein exceeding 8 per cent in 73 per cent of a material embracing 83 cases. As regards the patients in whom the albumin fraction is under the lower normal limit the hypoalbuminemia must probably be explained as a compensatory phenomenon, secondary to the hyperglobulinemia. Chester has reported two cases of hypoproteinemia in myelomatosis and comes to the conclusion that, if the patients are kept under observation for a sufficiently long time, hypoproteinemia will regularly be noted *ante mortem*. This assertion is refuted by Fellmer and Fowler (1937/38), and it likewise appears from the determinations of serum protein in the present material that premortal hypoproteinemia is rarely seen, while on the other hand a falling tendency in the total proteins may be noted *sub finem*. In Cases 2, 9, 11 and 12 such reduction of the total proteins was observed, while the numerical value of the A/G quotient showed no regular variation. In serial investigations Gormsen noted a fall in total protein, albumin and globulin during the progress of the illness. We found hypoproteinemia only

in one case, No. 4. In Cases 5, 7, 8, 10, 13, 14, 15 and 16 the total protein values were fairly constant all the time up to death.

For demonstration of B-J proteins in serum we have in part employed the boiling test, which is, however, inadequate because this method does not enable us to distinguish between B-J proteins and euglobulin. From the urine analyses, however, we can obtain information respecting the presence of B-J proteins in the serum. According to Moore and coworkers, B-J proteinuria signifies that these proteins are present in the blood, since that substance cannot be synthetized in the kidneys. A negative B-J reaction in urine, however, does not exclude the possibility of these proteins being present in the serum, seeing that complex, non-filtrable components of B-J proteins may there be formed. By analysis of the urine it has been established that *16 of the 17 patients examined had B-J proteins in the urine, and thereby we have proof that B-J proteins were present in the serum of these patients.* The frequency of B-J proteinuria is stated in the literature to be from 33 to 80 per cent (Gormsen, Bayrd and Heck, and others).

The formation of serum protein in immunisation has been studied by Björneboe (1943) and by Björneboe and Gormsen (1943). The increase in the quantity of globulin found (by Henriques and Klausen's method) after immunisation was identical with the rise in antibody protein, and the antibody formation and plasma cell proliferation were parallel phenomena. Sabin showed in 1939 that globulin is formed in the reticulo-endothelial system, and Landsteiner and Parker (1940) proved that fibroblasts formed proteins identical with, or closely related to the serum proteins. Thus there is evidence for the view that plasma cells produce protein, and the immature plasma cells in myelomatosis show cytochemical signs of active formation of proteins (cit. Olhagen). The more marked the cellular atypia is in myelomatosis, the higher will be the content of serum globulin. Continued cytochemical and electrophoretic studies will lead still a step farther towards the elucidation of the formation of serum protein.

Hypercalcemia in myelomatosis is explained by some authors as the result of a primary anomaly in the calcium metabolism. It is doubtless more probable that the hypercalcemia is partly due to the general osteoporosis, which, again, may come from the patients having often been confined to bed for a long time, and that it may partly be ascribed to pressure atrophy of the skeleton caused by the myelomas. *A priori*, the hypercalcemia might also

be supposed to be a consequence of the frequently occurring hyperproteinemia. On inspection of Table III it is seen that there is no absolute correlation between the values for serum calcium and the total proteins. The few investigations that have been published respecting the calcium excretion in urine in cases of myelomatosis show that the calcium content in the urine is often found to have risen, and balance tests reveal negative balance (Magnus-Levy, 1938). In normal kidneys only the ionised fraction of Ca and the Ca citrate (Ca-X) can be filtered, whereas the Ca proteinates and the colloidal phosphate complexes are retained. The dissociation of the Ca proteinates is dependent upon several factors, including the protein and Ca-ion concentration, as well as the A/G quotient. When the permeability of the glomeruli is increased, as we often find in myelomatosis, not only the ionized calcium and Ca citrate but also normally non-filtrable Ca fractions will be able to pass through the filter and consequently contribute to increase the excretion. The calcium incrustation of the tubules in myelomatosis is possibly of the same nature as in hyperparathyroidism, possibly the pathogenesis is identical with that of necrotizing nephrosis. The abundant content of phosphatase in the renal tissue may perhaps also come into play as a pathogenetic factor. The other known causes of deposition of calcium in the tubule cells may be excluded with a probability bordering on certainty. In two patients (Cases 3 and 8, Table III) the serum phosphorus was found to be considerably increased. In the other cases the phosphorus content was normal. The retention of phosphorus in the cases mentioned may be explained from the considerable impairment of the renal function in these cases (Table II).

The urea clearance does not give an exact expression for the quantity of filtrate in the glomeruli, as a varying portion of the filtered urea is reabsorbed in the tubules. In comparative investigations of urea and creatinine clearance there was found very good correlation between the results obtained by the two methods. According to Josephson (1945) there is good direct proportionality between the urea clearance figure and the quantity of glomerular filtrate. For clinical purposes the urea clearance consequently affords sufficiently exact information as to the renal function. On the basis of systematic investigations respecting the normal variations of the urea clearance values the lower normal limit has been fixed at 75 per cent of the normal (cit. Fishberg, p. 91). According to Smith, the urea clearance figure must fall below

50 per cent of the normal before retention of urea takes place in the blood. When the clearance lies between 20 and 40 per cent of the normal about half of the patients will show urea retention, and when the clearance is under 20 per cent retention of urea is regularly seen. The observations here submitted (Table II) accord well with Smith's figures. Eight of the myeloma patients had considerable retention of urea and low clearance values. In 5 patients there was seen a medium reduction of the urea clearance with or without increased urea content in the blood. When these figures are compared with Bannick and Greene's investigations respecting the blood urea concentration in myelomatosis (where 11 out of 12 patients had urea retention in the blood), it must be permitted to conclude that renal insufficiency is very common in cases of myelomatosis. *A glomerular lesion with reduced filtration* (determined by the urea clearance) *was found in 4—5ths of the patients in our material.* As test of tubular function we employed the dilution and/or concentration test in urine. *Tubular insufficiency* (Table II) *was noted in 12, or possibly 13, out of 16 patients, i. e., in about 3—4ths of those examined.* Bannick and Greene found fixed specific weight in the urine of all the patients examined, and they likewise noted reduced tubular excretion of phenol red. Both the glomerular filtration and the tubular reabsorption and excretion were impaired in the myeloma kidneys.

The relatively few autopsies made in cases of myeloma do not justify any far-reaching conclusions. The finding of indisputable glomerular changes without simultaneous mechanical occlusion of the lumen of the tubules in the material here submitted supports Randerath and Apitz's postulate that the genesis of the myeloma kidneys is to be found in a primary glomerular lesion and that the tubular degenerations are secondary. All the patients had proteinuria, which according to Randerath may be ascribed to functional changes in the glomeruli. Bell's assumption that proteinuria is expressive of histologically demonstrable glomerular lesions has been refuted by experimental histological studies of nephrosis. The question whether the glomerular changes are of arteriosclerotic nature or not cannot be answered, as our material is numerically inadequate.

If the results of the tests of renal function are compared with the findings in histological examinations, it is seen that there is good correlation between the functional derangements and the diffusion and degree of the anatomical changes.

Summary.

In the period 1933—1946 seventeen cases of multiple myelomas were treated in Med. Dept. B of the Rikshospital. The accumulation of cases in the last three years (Fig. 1) is regarded as merely a coincidence. The sex and age distribution is seen from Table I. It appears from the case-histories that pains in the back and in the extremities were the dominating initial symptoms.

In all the patients, except one, there was found hypochromic anemia of varying degree. In all cases the number of white blood cells was normal, while thrombocytopenia was noted in one patient. Proteinuria was present in all the patients, and with one exception they all had Bence-Jones proteins in the urine (Table II). A glomerular lesion with reduced filtration (determined by urea clearance) was observed in 4—5ths of the patients comprising the material and tubular insufficiency (investigated by the dilution and/or concentration test in urine) was found in about 3—4ths of those examined in this respect. The serum protein analyses (Table III) reveal in over half of the material hyperglobulinemia with low A/G quotient and increased content of total protein. On the basis of the urine analyses it can be regarded as established that 16 of 17 patients examined had Bence-Jones proteins in the serum.

The average time of survival was 1.8 years. The cause of death was cachexia in the great majority of cases.

The histological examinations in the seven cases where autopsy was performed revealed degenerative changes of the tubular cells. Glomerular changes were noted in 4 patients.

The findings in the few autopsies made seem to support the assumption that the glomerular changes form the primary factor in the genesis of the myeloma kidney.

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The Influence of Alcohol on Ketone Metabolism.¹

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Characteristic of the ingestion of alcohol and its distribution in the body is its rapid diffusion. This is of importance not only to the absorption, during which process 20 per cent of the quantity ingested will pass through the stomach, but also to the distribution in the organism, as alcohol will diffuse rapidly into the body fluids throughout which it will obtain a completely homogeneous distribution of equal concentration. The concentration obtained in the blood will be determined by the ratio between *absorption and elimination*. The excretion of alcohol is only of slight significance to the elimination, the tension in the urine being equivalent to that in the blood, and the quantity excreted by the lungs being very modest. Since practically the total amount of the ingested alcohol disappears in the organism it must be able either to burn or to be deposited in some way or other. Alcohol will first and foremost be eliminated through combustion. This is proved, amongst other things, by the fact that subsequent to the ingestion of alcohol the respiratory quotient will decline towards the respiratory quotient of alcohol, viz. 0.67. It never reaches this low level, however, as alcohol will never undergo combustion alone, but always simultaneously with other substances (Widmark (1930)).

Alcohol exerts an economizing effect on other nutrients which

¹ This investigation was performed with the support of Miss P. A. Brandt's Bequest.

is evident from the fact that the development of calories in persons who are fed certain quantities of fat, carbohydrate, and protein equals that of other persons to whom a certain quantity of alcohol is administered in addition to the same mixed diet.

The peculiar thing about the position of alcohol in the metabolism is that its oxidation always takes place at a constant maximum rate (Widmark (1930)).

Like most other substances, alcohol is preliminarily transformed in the liver where it undergoes primary partial oxidation into acetic acid. This is an absolute condition for utilization by the organism of the high caloric value of alcohol. *The primary partial oxidation in the liver regulates the extent to which alcohol can be oxidized* (Lundsgaard (1937)).

In undergoing combustion alcohol, as mentioned before, replaces isodynamic quantities of other food elements and exerts an economizing effect on the metabolism of these. Computations reveal that the combustion of alcohol is able to cover 70 per cent of the basal energy requirement of the organism.

Because of these properties alcohol has been turned into clinical use, in former days in the treatment of diabetes, and in such cases where patients, owing to their disease, have been unable to ingest ordinary food.

About the therapeutic use of alcohol Knud Ove Møller (1941) writes, amongst other things, that the therapeutic use of alcohol has been much disputed through the times, frequently from points of view more propagandistic than objectively medical. That is also the impression one gets by reading for instance the literature on the formerly so much discussed use of alcohol in diabetes mellitus.

Joslin (1935) says about the employment of alcohol in diabetes mellitus that in no other disease would the employment of alcohol appear to be more useful or more justifiable. The author does not often give it to diabetics, however, because of 1) the (admittedly rare) occurrence of alcoholic neuritis, 2) the danger of habituation, 3) the danger that lay people may mistake insulin shock for alcoholic intoxication, and 4) the author's personal disapproval of the use of alcohol.

Particularly in the clinical literature of some decades past records are available of a number of investigations into the effect of alcohol on the excretion of ketone bodies and sugar in diabetic patients.

Neubauer (1906) determined urinary excretion of acetone, B-oxybutyric acid, and ammonia in diabetics. He found that a dietary supplement of alcohol was ineffectual in mild cases with slight acidosis, whereas a distinctly favourable effect was observable both on the acidosis and the glycosuria in more severe cases. The diet used by the latter is described as one deficient in carbohydrate, but the exact quantities of protein and fat are not specified, nor is there any indication as to whether or not these quantities were kept invariable, in consequence of which one cannot rule out the probability that possible variations in the composition of the diet might have been responsible for the results obtained.

Benedict & Török (1906) kept diabetics on accurate mixed diets. For test purposes they substituted part of the fat by isodynamic quantities of alcohol, whereby they succeeded in reducing both the glycosuria and the acidosis. They performed analyses for the excretion of acetone alone, and not for B-oxybutyric acid, which renders these results less valuable. Their determinations of the excretion of ammonia, however, reveal evidence of a decided diminution of the acidosis when alcohol is substituted for part of the dietary fat.

Stäubli (1908) examined a diabetic with moderate acetonuria whom he first placed on a high fat diet to which he subsequently added alcohol, viz. over a period of two days 1750 cc of wine containing 10 per cent of alcohol. At first a considerable decline was noted in the excretion of both acetone and γ -oxybutyric acid, but then followed a rise to levels higher than those observed at the initiation of the experiment.

Higgins, Peabody, & Fitz (1916) performed extensive experiments on themselves. They first provoked an acidosis with ketonuria by ingesting a carbohydrate-free diet for three days. On the fourth day they ingested a fairly large quantity of alcohol in addition to the diet. They did not succeed in demonstrating any diminution of either the acidosis or the ketonuria.

An identical result was obtained by Allen & Wishart (1922). They examined the influence of alcohol on two diabetics. It is their opinion that alcohol produces hyperglycosuria as well as a slight rise of ketonuria when added to the diabetic diet in quantities exceeding the caloric tolerance.

von Noorden & Isaac (1927) report that alcohol has a lowering effect on the glycosuria and the ketonuria in diabetic patients;

particularly on the ketonuria in severe cases. Furthermore, the effect on the ketonuria is most pronounced on days of fasting. Leclerc. (1922) observed hyperglycemia in patients with severe diabetes in consequence of adding excessive calories to the diet in the form of fat or alcohol.

Strouse, Soskin, & Vidgoff (1935) find no effect of alcohol, either harmful or beneficial, in the diabetic diet.

Many other investigations of a similar nature have been carried out. As will be seen, results have been highly conflicting. The most common conception has been that alcohol has a favourable effect on a diabetic condition. However, present-day treatment of diabetes makes the use of alcohol quite unwarranted, for which reason the problem has receded into the background in modern times.

The question is, however, of no slight theoretical importance. So far no conclusive evidence has been produced of the effect of alcohol, if any, on a ketosis. In previous investigations an essential error was committed in performing tests for urinary excretion, and not for blood concentration. The fact that part of the ketone bodies, which are supposed to be useful for the organism, are reabsorbed by the kidneys, makes an estimation of the urinary content a too inaccurate method for assessing changes of ketone metabolism in the body.

The Writer's Investigations.

Technique. Jacob E. Poulsen's micro method was employed for determining blood ketone concentration. For further particulars about this test cf. Jacob E. Poulsen's dissertation (Copenhagen 1941), and Warming-Larsen (1947) in which this method and laboratory checks and controls with known quantities of ketone bodies are described. The aceto-acetic acid + acetone and B-hydroxybutyric acid were determined separately and the total amount of ketone bodies is expressed as B-hydroxybutyric acid.

When studying the effect of alcohol on ketonemia one will at once be confronted with a practical difficulty. Alcohol and ketone bodies will prove to be reciprocal sources of error in analyses for one of these substances. In the presence of a ketonemia a concentration of alcohol, if any, will be measured too high, and vice versa a ketonemia will be measured too high if the person has ingested alcohol.

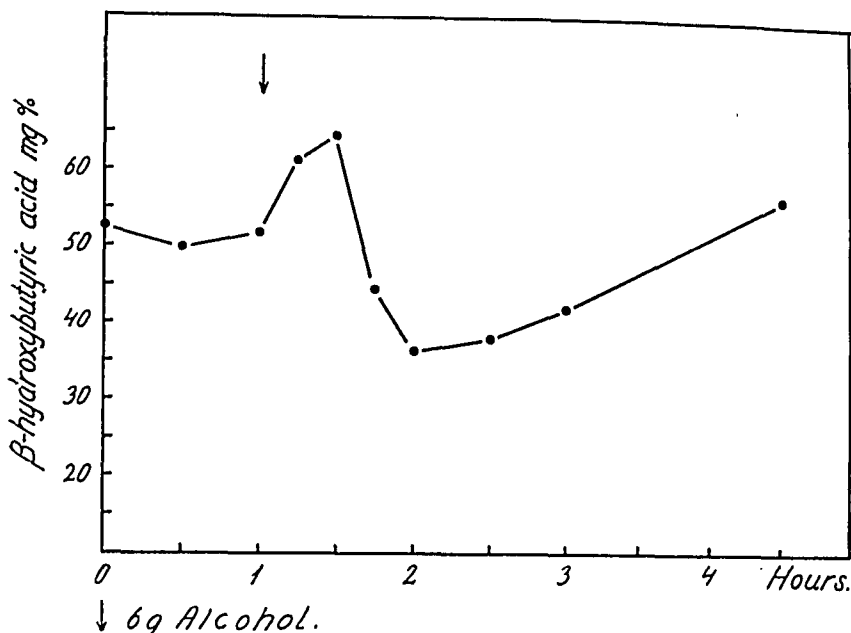


Fig. 1.

Bearing in mind that alcohol is oxidized at a constant maximum rate, I have endeavoured to administer alcohol in such small doses that the elimination (combustion) could approximately keep pace with the intake. This would result in the alcohol being oxidized without the occurrence of a concentration of alcohol in the blood.

An inanition ketonemia was produced, and after ascertaining the concentration of ketone bodies through repeated measurements within a fairly short period, I administered alcohol.

The experiments recorded were performed upon non-diabetics to whom alcohol was administered following three days of fasting.

This curve reveals an *increase* of the inanition ketonemia immediately after the intake of alcohol, followed by a notable *decrease* below the original level.

In order to find out what was responsible for this increase — which might be due to some specific phenomenon or merely to the fact that even this small dose of alcohol transitorily might have produced a certain concentration in the blood which would lead to erroneous determination of ketone bodies — I made other experiments in which 2 g of alcohol were given every twenty minutes so as to protract the absorption.

Four experiments in all were performed in this way. They

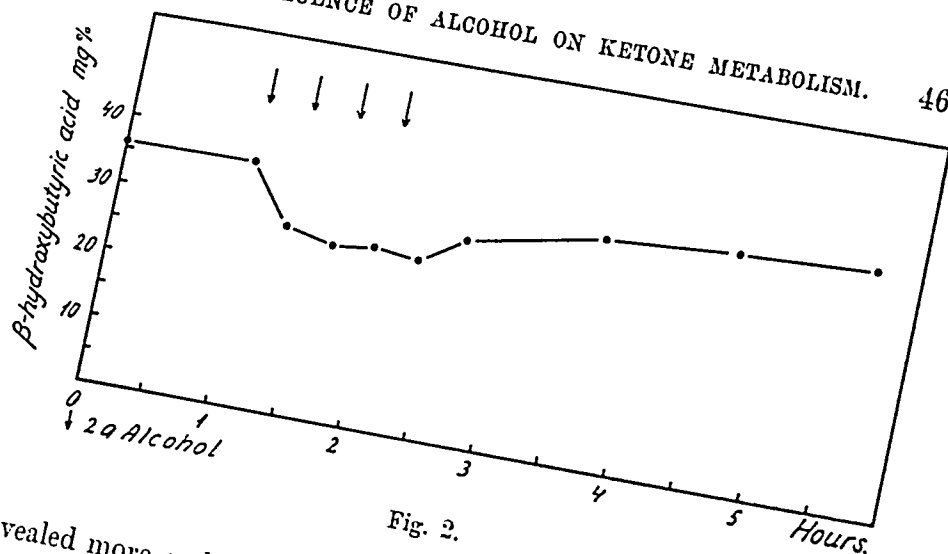


Fig. 2.

revealed more or less the same result: The positive »jag« has now disappeared or is, at least, very small, but even now a rather distinct increase of ketonemia is observable.

Finally, three experiments were made in which alcohol, in a 0.9 per cent solution of sodium chloride, was administered by continuous intravenous injection at the rate of 6 gm per hour.

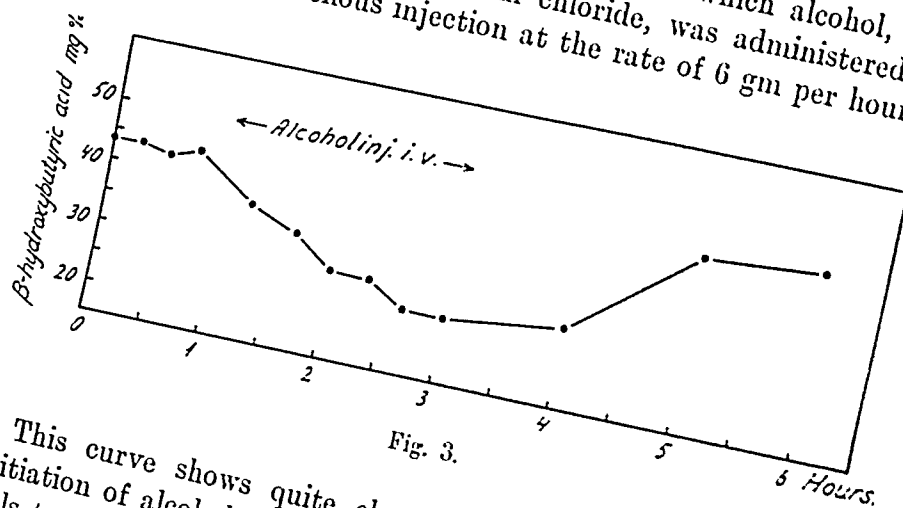


Fig. 3.

This curve shows quite clearly that immediately after the initiation of alcohol injection the concentration of ketone bodies falls to a considerably lower level where it remains until shortly after discontinuation of the injection, whereupon it rises to its initial level.

There can be but one explanation for these results. The combustion of alcohol must have an economizing effect on the fat oxidation which will enable the liver to reduce its ketogenetic activity. This is in accord with the existing belief that a ketonemia

occurs when an organism is forced exclusively or predominantly to oxidize fats, with the result that the high concentration of ketone bodies obtained through the intensified ketogenetic activity of the liver will facilitate the oxidation in the tissues (Lundsgaard (1941)).

Consequently, alcohol is an antiketogenic substance. The view of earlier investigators that antiketogenic substances were identical with glycogenetic substances can no longer be maintained. This is shown clearly by these results. An antiketogenic substance means a substance which economizes the fat metabolism and, consequently, the ketogenesis by furnishing calories to the extra-hepatic tissues.

These experiments can safely be regarded as confirmatory evidence of the fact that alcohol has a lowering effect on an inanition ketonemia, which justifies the administration of small doses of alcohol to patients who for some reason are unable to ingest ordinary food and, in consequence, develop acidosis.

This observation will hardly be of any practical importance in the treatment of diabetes, as it being so well organized at present through diet and insulin therapy that nothing further can be said to be required. However, moderate doses might perhaps yield gratifying results in individual cases, particularly in cases of less severe diabetes in elderly people who are on the verge of requiring insulin therapy.

The essential significance of these experiments must, however, be said to be of a theoretical nature, giving support from a new angle to the theory that ketone bodies are *normal* products of metabolism the formation of which increases with the amount of fat metabolized.

Summary.

Alcohol has proved to be a source of error in analyses for ketone bodies according to the method here employed. The fact that alcohol is oxidized at a constant maximum rate makes it possible, however, by continuous intravenous injection, to create a balance between the combustion and the intake and thus prevent that an essential concentration appears in the blood at any time.

On slow continuous intravenous injection of alcohol in a 0.9 % sodium chloride solution the blood ketone concentration in an organism with inanition ketonemia was found to fall to lower

levels for the duration of the injection, whereupon it rose rapidly to its original level when alcohol was administered continuously in doses which did not quite cover maximum capacity of the organism for oxidation over the same period of time. There is hardly any other explanation for this than the assumption that alcohol exerts an economizing effect on the fat oxidation, in consequence of which the originally high blood ketone concentration, which was required for enabling the organism to oxidize ketone bodies to such a large extent, can now be regulated down on a lower level as long as alcohol furnishes a considerable part of the caloric requirement.

These studies are quite in keeping with the view that an anti-ketogenic substance is merely a substance which by its own combustion exerts an economizing effect on the fat combustion, and in reality they show quite clearly that antiketogenic substances are not identical with glycogenetic substances.

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Study of Streptococcal Agglutination.

With Special Reference to the Specificity and its Relation to the Erythrocyte Sedimentation Rate.

By

A. BÖNI and STEN WINBLAD.

(Submitted for publication February 4, 1948.)

Streptococci belong to those bacteria that on account of their chain formation readily exhibit spontaneous agglutination. In view of this fact it was for a long time difficult to produce sera agglutination that could be considered as a specific antibody. By means of an absorption process, Griffith was able to produce type-specific sera and to utilize the agglutination as a means for determining the type specificity of hemolytic streptococci.

However, Nicholls and Stainsby (1931) as well as Dawson, Olmsted, and Boots (1932) showed that sera taken from rheumatoid arthritic patients agglutinated such strains of streptococci, as did not agglutinate spontaneously. Whilst the first-mentioned authors believed that this agglutination was peculiar only to one special streptococcal strain the others showed that it was manifestable in various different strains of hemolytic streptococci.

One condition for such agglutinative reaction was, however, that the culture did not agglutinate spontaneously and that it was not permitted to grow for more than 14 hours. A rather large number of authors established the occurrence of such agglutination in patients suffering from rheumatoid arthritis (Meyers, Keeper and Oppels; Cox and Hill; McEwen, Bunim and Alexander; Straub and Hartung; Lewinthal; Packalén, Cecil and de Gara). Generally speaking, about 60 per cent of all patients with rheumatoid arthritis exhibit such agglutinative reaction.

Of late years Kalbak has again drawn attention to this agglutinative reaction which was manifestable in 76 per cent of an extensive rheumatoid arthritic material, and in 90 per cent of a material consisting of uniformly and clearly diagnosed cases of rheumatoid arthritis, whilst only 10 per cent of the cases with rheumatic fever exhibited such agglutinative reaction. Also Winblad was able to observe such agglutination in patients with rheumatoid arthritis. In 206 cases he was able to show that 68 per cent had a titer of $1/20$ or more. An interesting observation was made by Winblad & Edström who established the absence of agglutination in the acute stage of rheumatic fever, but the presence of increased agglutination occurring initially after about 4—6 months. This argues for the assumption that the agglutination is a serological late-phenomenon and that it is bound to the chronic phase of the rheumatic disease.

Whilst all these agglutinations are shown against living, briefly incubated (approx. 12 hrs.), hemolytic streptococci of the A-group, Thulin proved that it was possible to show agglutination also against an autoclaved antigen, which he designated O-antigen (in conformity with *Salmonella-Coli* serology). Thulin obtained agglutination against this antigen in sera from patients suffering from nephritis and polyarthritis, both in the acute and in the chronic stages. Thulin could show, also by absorption tests that this antigen differed completely from that of living streptococci.

In the discussion of these agglutinins the question is often raised by clinicians whether the agglutination is definitely due to a specific antigen-antibody reaction and consequently justifies the conclusion that, in case of a positive result of the reaction, streptococci of A-group play a part. Certain objections might be brought forward against the specificity of this reaction. As already pointed out above, these antibodies appear rather late after the occurrence of streptococcal infection. Approximately simultaneously it is possible to establish an increase of the β -or γ -globulins in the serum of patients with rheumatoid arthritis (Böni). These globulins might possibly be associated with or related to specific antibodies (Wuhrmann et al.) and might consequently be responsible for the positive agglutination and cause an illusory specific reaction. Furthermore, we know that this pathological globulin fraction plays a part in the erythrocyte sedimentation rate (E. S. R.), but not before the disease has broken out. The question might be given a more general construction: does an in-

creased non-specific agglutinative reaction occur in all chronic infectious diseases exhibiting elevated E. S. R.?

In view of the above we made the following examination: We determined the agglutinative power of hemolytic streptococci in serum from patients with chronic pulmonary tuberculosis.¹

We selected this disease on purpose, firstly because, as mentioned above, there occurs an increase in the globulin fraction similar to that peculiar to rheumatoid arthritis, and secondly, because, as far as elevated E. S. R. is concerned, it ranks with the last mentioned disease. With the serum of these tuberculous patients we produced agglutination against living, briefly incubated hemolytic streptococci by the method used by Dawson and by Kalbak (in this paper called L-agglutination. L = living antigen) as well as against autoclaved antigen in the manner suggested by Thulin (O-agglutination).

Technique.

L-agglutination.

We adopted the method used by Kalbak and utilized Kalbak's strain SF 130 of group A, type 1.

Broth I. 750 g finely minced beef is added to 1.5 litres of tap-water and kept over night in a refrigerator. The broth is then boiled 15 minutes, filtered through paper and alkalized with 5 N NaOH to a pH of 8.0. To this 500 ml broth 10 g Neopepton and 4 g sec. sodium phosphate are added. This mixture is boiled and its pH adjusted to 8.2, subsequent to which it is passed through filter paper and autoclaved for 20 mins. at 120° C.

Broth II. Instead of the above mentioned neopeptone and sodium phosphate, 10 g bactopecton and 5.6 g NaCl are added to the broth whose pH is adjusted to 8.0. The broth is then boiled and its pH adjusted to 8.2, subsequent to which it is again filtered through paper and filled into various sterilized flasks, which are then autoclaved 20 mins. at 120° C. The pH value is finally adjusted to 7.6—7.8. Broth I is then inoculated with the culture and allowed to grow 6 hours, after which Broth II is inoculated with Broth I and allowed to grow 12 hours. These living cultures are utilized as antigens in the agglutination. The streptococcal culture thus produced is then utilized for the preparation of a diluting series comprising serum from patients and 0.3 % NaCl in the proportion 1/40—10/640. For the evaluation of the degrees of agglutination we have also adopted the definitions suggested by Kalbak.

¹ We beg to express our appreciation to Dr. Eilert Selander for placing serological examination material to our disposal.

Degree 0:

No agglutination. When the flask is shaken gently, a diffuse rising of the sediment.

Degree 1:

No real agglutination. When the flask is shaken diffuse clouds of small sedimental particles rise. On the whole the liquid is cloudy.

Degree 2:

Real agglutination. The sediment now breaks up into medium-sized particles when agitated. The liquid is clear.

Degree 3:

The sediment now consists of large partially coherent particles. The liquid is clear.

Degree 4:

The sediment consists now of a large solid slab that is not easy to break up. The liquid is clear.

We look upon degrees 0 and 1 as being negative. We stipulate at least degree 2 in the first test tube, if the agglutination is to be considered positive. We think it possible, however, to be somewhat generous concerning intermediate values such as 1—1—0—0—0 or 1—1—1—0—0, *i. e.* repeatedly doubtful agglutination. We have been bold enough to designate this agglutination 1/20, although we are well aware of the fact that in so doing we are obliged to be somewhat generous, but to so small degree as might be considered negligible. Kalbak also described the occasional appearance of irregular agglutination, designated by him as «negative, atypical agglutination», and which manifests itself *inter alia* by a presence of agglutination in the last 2—3 tubes but an absence in the first tubes, or by the fact that the agglutination pursues an irregular course. This question will be duly dwelt on further down when it appears in the study.

O-agglutination: We utilized Thulin's culture A I, belonging to Group A. After 18 hours' growth on pancreatin-digested placenta broth adjusted to pH of 7.5, the culture was centrifuged out and washed with an NaCl-phosphate buffer at pH 7.5, after which it was autoclaved 2 hours at 120° C. To culture thus autoclaved was then washed three times with the phosphate buffer in question.

The pancreatin broth is prepared in the following manner: 1½ kg ground placenta is mixed with 3 litres tap-water and allowed to stand 8 hours at 37° C, after which it is autoclaved 1½ hours at 110° C. and allowed to cool. A teaspoonful of precipitated chalk and 60 ml chloroform are then added to the mixture which is vigorously stirred and allowed to stand 5—6 days at room temperature. Should meat particle rise to the surface, chloroform should again be added. The mixture is then again autoclaved 1 hour at 110° C, filtered through double paper, after which it is re-autoclaved. From this mixture the final broth is prepared in the following manner: Two parts by volume of the mixture

are added to one part aqua dest. Three g NaCl, 2 g sec. sodium phosphate and 1 g glucose are then added per litre to the mixture. The broth must be boiled and its pH adjusted to 7.6—7.8 after which it is again boiled for 10 minutes and passed through double filter-paper and autoclaved 20 minutes. The broth is then ready for use.

The registered degree of agglutination coincides exactly with that of L-agglutination. Concomitantly with the examination of sera from tub. patients also sera from patients with rheumatoid arthritis were examined under the same conditions. These arthritic sera constituted a control material.

1. The Frequency of Manifestable Agglutination in Sera from Tuberculous Patients.

From 92 different sera from an equal number of patients positive L-agglutination could be established in 9 cases, *i. e.* in 9.8 per cent. These agglutinations were however rather weak and exhibited a titer of 1/40. The figures obtained, 9.8 per cent, coincides roughly with that of normal material examined by earlier investigators (Cecil, Nicholls and Steinsby, 2 p. c.; Olmsted and Boots 2.4 p. c.; Keefer, Meyers and Oppels, 2.2 p. c.; Packalen, 11 p. c.; Kalbak, 1.5 p. c.). Although the figure does not coincide exactly, it is obviously but slightly higher, so that one may almost speak of similar frequency; it is also known that in tuberculous bronchitis there often occurs complex- or superinfection of hemolytic streptococci (Westergren and al.). This consequently justifies a percentage slightly higher than in normal material. For this reason a slight increase may be possible in the degree of agglutination of tuberculous material as compared with that of normal serum.

In the simultaneously examined sera from 30 cases of rheumatoid arthritis, agglutination could be manifested in 20, *i. e.*, 66 per cent., a number coinciding very well with Winblad's material. This is definitely presumptive of streptococcal agglutination being an exceptional phenomenon in the disease in question.

In the O-agglutination in serum from tuberculous patients agglutination was shown in 2.1 per cent of the cases, which coincides with that which Thulin proved to be peculiar to normal material (see Fig. 1).¹

¹ Further investigations on the presence of O-agglutinins in sera from healthy people have shown that the result may vary considerably in different materials. One of us (S. W.) has found as much as 50 % positive reactions in sera from healthy persons (blood donors). This discrepancy probably depends on some inconsistency of the antigen.

2. The Relationship between the E. S. R. and the Streptococcal Agglutination.

This relationship will be apparent from Fig. 2 from which is to be read that the average mean value of the E. S. R. with positive L-agglutination was 46 mm/hr., with pos. O-agglutination, 10 mm/hr., and with negative L-agglutination, 32 mm/hr.

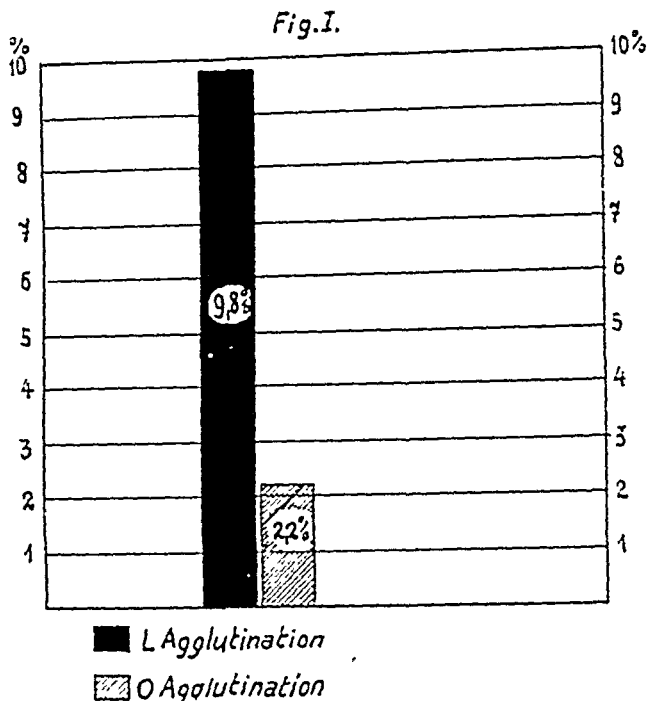


Fig. 1. Percentage of L and O-agglutination in the serum of 92 tuberculous patients.

As mentioned above, it was our desire to determine whether the increase in the globulin fraction occurring also in chronic pulmonary tuberculosis and involving elevated E. S. R., possibly causes a non-specific agglutinative reaction. *It would however seem as if the elevated agglutination index is not due to an elevated globulin value but that this agglutination is probably to be ascribed to the infection by hemolytic streptococci.* The average of the pos. L-agglutination is certainly higher than in the neg. agglutination, but in view of the dissimilar frequencies of the two groups, there is no essential statistical difference between the two groups. In the different sera it was frequently observed that cases with elevated

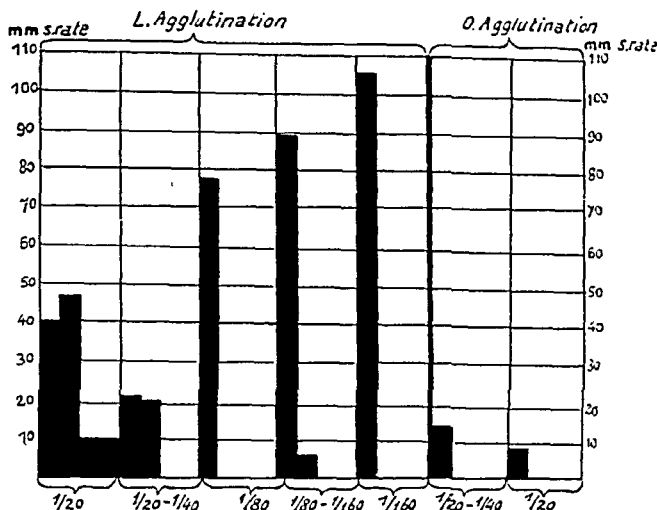


Fig. 2. Occurrence of the L and O-agglutinations in the serum of tuberculous patients and its relationship to the E. S. R., ordinate: sedimentation value, abscissa: agglutinative power.

E. S. R. exhibited no agglutination (see Fig. 3). The agglutination in the tuberculous cases may possibly be a result of an admixture of hemolytic streptococci in the bacterial flora in the bronchus.

O-agglutination was apparent in cases with especially low E. S. R., but it is difficult to decide whether this was just a coincidence or whether other factors played a part.

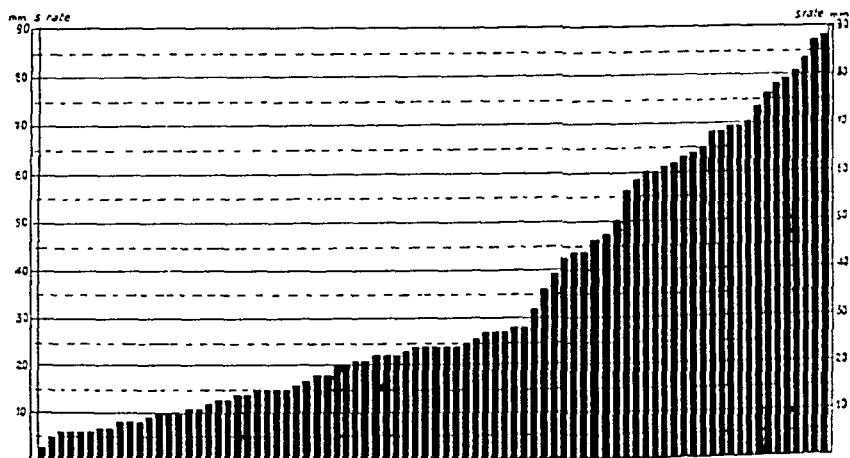


Fig. 3. The sedimentation values in the negative agglutination specimens in the serum of tuberculous patients. This diagram shows distinctly that no positive agglutination occurs even with the high sedimentation values.

3. Findings from »Negative, Atypical Agglutination». (Fig. 4).

In those cases in which no agglutination could be observed in the first or in the second tube but especially in the fourth and fifth tubes, we presumed the existence of a »negative atypical agglutination» according to Kalbak. The agglutinative degree of

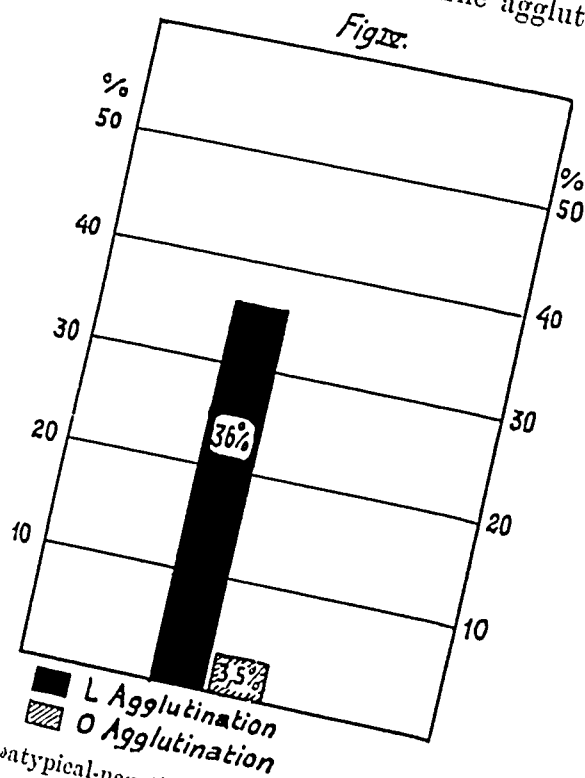


Fig. 4. Percentage of »atypical-negative» L and O-agglutination in the serum of 92 tuberculous patients.

these atypical agglutinations seldom reached the value 2. In the L-agglutinations, atypical negative reactions of this nature could be discerned in 33 cases (36 per cent) but only in three cases, in O-agglutination. Of particular interest is the fact that these atypical agglutinative tendencies could be manifested only in those tubes in which the dilution was 1/320 and 1/640. O-agglutination was more marked in its specific agglutination, and such negative, non-specific agglutination seems to occur less frequently in O-agglutination. An atypical negative agglutination of this character was observable also in sera from polyarthritic cases, but not so often as in sera from tuberculous patients.

One might be tempted to ask whether this negative atypical agglutination phenomenon might not be presumptive of the existence of a certain agglutinative tendency. The one and only assertion we wish to make here is, however, that such agglutina-

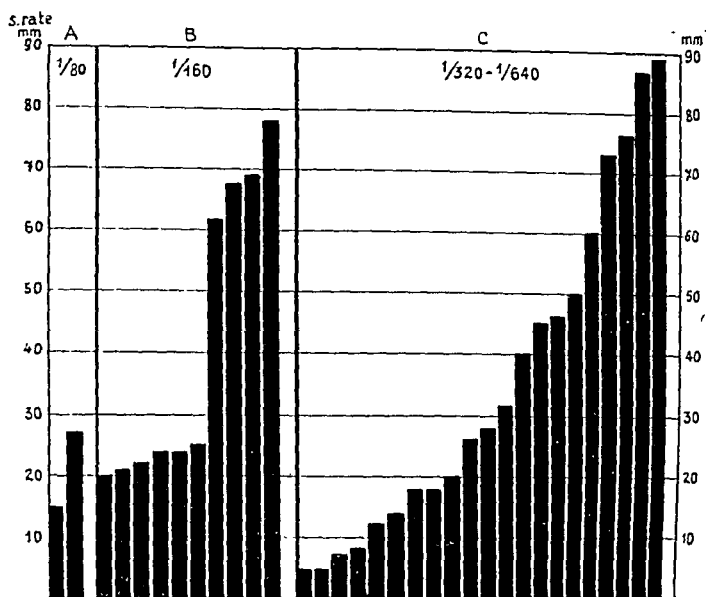


Fig. 5. The relationship between the «atypical-negative» agglutination and the E. S. R. in the sera of tuberculous patients.

a) Commencement of the «atypical-negative» agglutination with a 1 : 80 dilution of the sera.

b) Commencement of the «atypical-negative» agglutination with a 1 : 160 dilution of the sera.

c) «Negative-atypical» agglutination occurring only in a 1 : 320 and 1 : 640 dilution of the serum.

tion, though weak, is often observable in sera in which «typical agglutination» is rare.

This «negative, atypical agglutination» shows no relation to elevated or low E. S. R. (see fig. 5). There is probably no serological action here and the agglutination in question has probably no relationship to increased globulin values.

4. Relationship between L and O-Agglutination.

As mentioned above Thulin could show by means of an absorption process that the antigen causing L-agglutination is different to the one causing O-agglutination. In our examinations it

could be observed that in some of the sera of examined tuberculous patients, some of the O- and L-agglutinations did not always appear together.

Continued examination of these two antigens and the part they play in chronic polyarthritis is essential for the further elucidation of the agglutination phenomenon in sera from these patients.

Summary.

In order to examine the specificity of agglutinins (L-agglutination after Dawson and Kalbak, and O-agglutination after Thulin) sera were taken from 92 patients suffering from chronic pulmonary tuberculosis.

The examination showed that L-agglutination in these sera occurred in 9.8 per cent, and O-agglutination in 2.1 per cent of the cases.

It was not possible to establish any definite relationship between the occurrence of elevated E.S.R. and elevated agglutination titers. The agglutination ought thus not to be ascribed to an elevated value of serum globulins in the serum, but to an antigen emanating partially or entirely from hemolytic streptococci. In a rather limited control material of patients with rheumatoid arthritis L-agglutination could be manifested in 20 per cent of the thirty cases.

Negative, atypical agglutination occurred more frequently in L-agglutination than in O-agglutination. This is probably to be attributed to a certain tendency on the part of the culture to agglutinate spontaneously. This agglutination exhibited no relationship to elevated or low E. S. R.

L and O-agglutination may occur independently in different sera.

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On the Artificial Kidney V.

Some Experiences During the Study of Dialytic Treatment on Animals with Uremia Caused by Mercuric Chloride Poisoning.

By

NILS ALWALL, LEMBIT NORVIIT and A. M. STEINS.

(Submitted for publication March 5, 1948.)

The effectiveness of dialytic treatment of uremia after ligation of the ureters of a rabbit has been reported in an earlier paper, A. and N., 1947; thanks to repeated treatments the blood-non-protein-nitrogen (N.P.N.) level did not rise above 100—125 mg% in 138 hours.

In paper IV of this series we reported that — the cause of the above mentioned hemorrhage from the operation wound having been eliminated — we now have no difficulties in treating a normal animal repeatedly with dialysis of the blood during protracted heparinization.

In the following we report some of our experiences during our studies of dialytic treatment of uremia produced by experimental damage to the kidneys (mercuric chloride) of rabbits. We began this work in order to study the *therapeutic value of dialytic treatment* under these conditions. We encountered new methodological difficulties. In the following we report some results, negative and positive, that seem to be of interest in regard to dialytic treatment, among other things in regard to acute nephritis with its often general damage to the blood vessels and in regard to the uremic damage to the mucous membrane, all of which involves risks of complications in heparinization and in dialytic treatment

of patients. In this connection observations concerning the toxicological problem of experimental mercuric chloride damage to rabbits will only be mentioned briefly; we hope to return to this problem in another connection.

Control Material.

At first we tried using 30 mg mercuric chloride per kg body-weight per stomach tube in the manner described by Haam and Fine, 1932. However, the animals were badly affected, and had serious diarrhoea.

We then proceeded to examine the effect of mercuric chloride administered intravenously. Fig. 1 shows the effect of 2 mg mercuric chloride per kg bodyweight on blood N.P.N. and length of life; 5 animals out of 17 survived. The values for those mentioned first are marked by small circles. Three of the surviving animals showed a relatively small rise of the N.P.N., not above 150 mg%. Of the 12 dead animals, one died after 42 hours with an N.P.N. of 193 mg%, one only reached 280 mg%, while the rest all had higher values; four reached 400 mg% or more. 11 of the animals died within 42—132 hours after the mercuric chloride injection, while 1 survived 300 hours. Two animals have been excluded from the figure, having died 2 and 26 hours respectively after the injection.

Figure 2 shows the effect of 3 mg mercuric chloride. All the animals except 1 died within 52—120 hours after the injection, all of them at blood-N.P.N. levels above 300 mg%; one animal, whose curve is not wholly reproduced on the diagram, died after 316 hours at an N.P.N. value of 562 mg%.

As is well known, mercurial poisoning first causes an increase in the diuresis, which can then turn into anuria. The animal can die from anuria, but also from uremia in spite of the fact that the diuresis may have recommenced. With injections of these doses, the animal had diarrhoea to a less degree than after 30 mg per kg orally, and their general condition was less affected. The rabbits had continual free access to water and food.

Fig. 3 gives the change of weight as a percentage of the initial value after 2 and 3 mg mercuric chloride respectively. As a rule the weight of the animals was checked every day.

To save space the diagram has been limited to 200 hours after the injection of mercuric chloride. Therefore the following information is missing: a) *from the group 2 mg/kg; surviving animals:* 1 animal 206 hours (— 9 %), 210 h. (— 11 %), 255 (— 11 %), 288 (— 12 %);

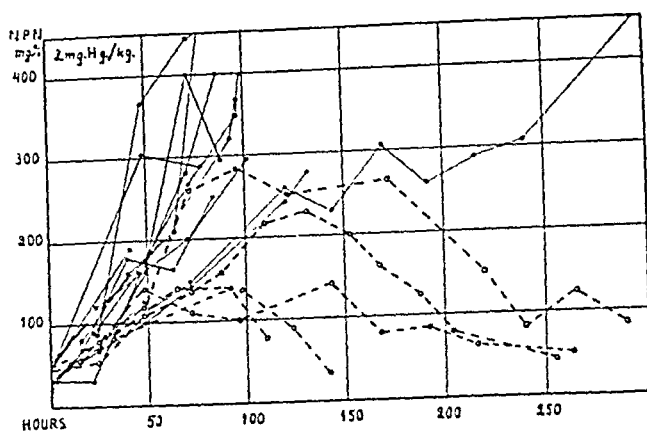


Fig. 1.

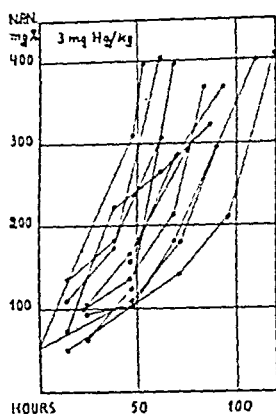


Fig. 2.

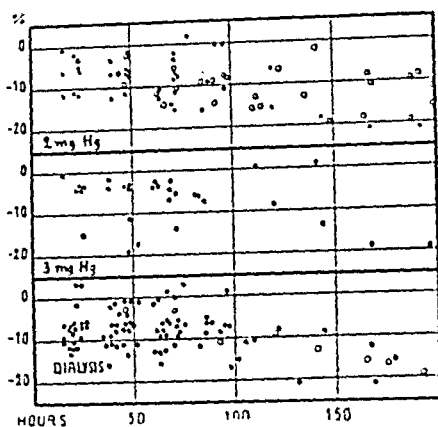


Fig. 3.

(Small circles, dotted lines: Survivors.)

for another animal: 213 (— 15 %), 234 (— 10 %), 257 (— 15 %), 281 (— 13 %); for two more animals: 256 (— 5 %) and 288 (+ 3 %) respectively; *Dead animals*: one animal: 240 hours (— 28 %). b) *from the group 3 mg/kg*: one animal: 240 hours (— 19 %), 264 (— 27 %) and 288 (— 31 %).

It appears that as a rule the weight is reduced and that this reduction remains for a long time even in animals surviving the poisoning. There does not seem to be any difference between the animals that had 2 mg mercuric chloride and those that had 3 mg per kg body weight.

There is no definite relation between on the one hand the length of life of the animal after the injection of mercuric chloride and the initial weight or the reduction of weight on the other, whether expressed as absolute numbers or as a percentage of the initial weight.

In order to make up for the animal's loss of weight we tried in some cases to augment the fluid they took themselves by a parenteral supply of fluid. During anuria the increase in weight caused by the fluid remained, but the rabbits generally lost weight again in combination with diarrhoea. The fluid therapy possibly improved the conditions of the animals in some cases, but on the other hand our experiences showed that there is a risk of overdosing. The question of the value of fluid therapy under these conditions is therefore left open for the time being.

Post mortem examination after mercurial poisoning showed typical macroscopic and microscopic changes in the kidneys. The microscopic examination has kindly been carried out by Professor C. G. Ahlström, M. D.

Material Treated with Dialysis.

As a rule the same number of control animals and animals intended for dialytic treatment were injected simultaneously with mercuric chloride. Therefore the control and experimental animals were practically uniformly distributed within the same space of time. The animals treated with dialysis also had free access to food and water, but during dialysis and the preparations for this treatment the animals generally took little food. And, in addition to the dialytic treatment itself, the operation and the cannulae, generally left in the wounds for several days running, had an unsettling effect on the experimental animals.

Dialytic treatment was carried out with the technique described in an earlier paper. As a rule the length of the cellophane tubing was 0.65 meters and the rate of flow of the blood 2—3 litres per hour. No clot-air-trap. The dialyzer was placed some 15 cm below the heart of the animal to attain suitable hydrostatic pressure. The weight of the animal was checked several times during the experiments; no change of weight as a consequence of the dialysis was found as a rule. At the end of the dialysis the animals were generally given most of the blood in the cellophane tubing, which gave them a certain surplus above the restitution of the samples of blood taken. In the event of profuse haemorrhage with sinking blood values, transfusions have been administered. The average dose of heparin is reported in the following. We have rarely found any infection in the wound, even when the cannulae have been left in the wound for several days.

The first dialytic treatment has as a rule been performed on the third day after the injection of mercuric chloride, and repeated every day when the blood-N.P.N. has increased, even in cases, where diuresis has recommenced. We adhered to this programme, even after we discovered that the control animals could survive several days' rise of the N.P.N. — in order to get experience of dialytic treatment and the effect on the animals of the heparinizing involved by dialytic treatment. Whether a better *therapeutic* result could have been achieved by interrupting the dialytic treatment earlier will not be discussed here.

In all, 27 mercuric chloride poisoned animals have been treated with dialysis; one had been given 4 mg mercuric chloride, five had had 3 mg and the rest 2 mg/kg. Fig. 3 gives the changes of weight in all these animals during the time of observation. The following values for a surviving animal have not been recorded on the diagram: 213 hours (— 19 %), 236 (— 18 %), 248 (— 19 %), 268 (20 %).

Fig. 3 thus contains the changes of weight for the animals, treated with dialysis, that had been given 2, 3, and 4 mg mercuric chloride respectively. As mentioned the corresponding values for the control animals are given in two figures: fig. 1 (2 mg/kg) and fig. 2 (3 mg/kg); as the changes of weight of these two groups appear to correspond it has seemed permissible to record all the animals treated with dialysis on one diagram. There do not seem to be any essential differences between the controls and the animals treated with dialysis.

Table 1 contains essential particulars concerning the material.

The date of the experiment has been recorded as it might be possible that the resistance of the animals may change with the season. The results do not seem to indicate such an assumption. On the other hand it may be possible that the changes, if any, have been compensated by the improvement in the technique that comes with a practised routine.

Initial weight. The average weight of this group was 2.70 kg and 2.44 kg for the control animals. Thus somewhat bigger animals were chosen for dialytic treatment.

Length of life. The average length of life after the injection of 2 mg mercuric chloride per kg is 89.5 hours (corresponding value for the control group 108.0) and 87.3 hours after 3 mg/kg (100.5 for the control group).

Out of 21 animals that had been given 2 mg mercuric chloride per kg and treated with dialysis 1 survived; in the corresponding control group 5 survived out of 17. All the animals in both groups died after 3 mg/kg.

Thus the chance of survival after the injection of mercuric chloride (length of life) of the animals has diminished in this group. When discussing complications we shall return to the explanation of this.

Length of dialytic treatment. The 27 animals were treated with dialysis during 328 hours in all, on 61 different occasions. Each treatment has lasted $5\frac{1}{4}$ hours on the average. The most prolonged

Table

Nr.	Date	Rabbit Nr.	Initial weight	Hg Cl ₂ mg/kg	Length of life hours	Dialysis treatment hours	N.P.N. in blood before/after dialysis treatment, mg %
1	27/5	36	2,350	2	75	6.15, 2.30	210/71, 190/90
2	31/5	55	2,372	2	184	5.30, 3.15, 2.40, 4.15	154/50, 95/63, 200/154, 200/83
3	10/6	64	2,270	2	86	4.00, 6.00, 3.45 ..	143/83, 242/80, 143/87....
4	10/6	24	2,720	2	108	4.00	143/91
5	11/6	38	2,900	2	103	6.45, 6.00, 5.30 ..	100/61
6	23/6	31	3,080	2	44	2.30	210/118, 143/80, 146/60...
7	3/8	67	2,625	2	60	5.00	125/100
8	6/8	44	3,715	2	survived	6.00, 6.00, 5.45 ..	166/100
9	30/8	91	2,835	3	65	6.00, 2.45	174/83, 182/100, 166/80...
10	8/9	68	2,700	3	86	6.00	160/87, 143/125
11	15/9	72	2,540	4	76	5.00	190/135
12	30/9	106	2,735	3	112	6.00, 6.00	133/100, 160/125
13	20/9	111	2,780	3	103	6.00	235/133
14	27/9	115	3,180	3	82	8.00, 6.30, 7.00 ..	228/111, 182/100, 210/143.
15	23/9	85	2,710	2	58	7.45, 7.15, 7.00 ..	286/154, 250/125, 222/143.
16	23/9	87	2,575	2	96	6.00, 7.45	235/143, 250/166
17	4/10	79	2,780	2	77	6.00	153/91
18	4/10	88	2,805	2	42	6.00, 7.00, 6.00 ..	125/90, 160/83, 148/91....
19	5/10	80	3,005	2	184	5.00, 5.00	133/83, 125/91
20	12/10	118	2,330	2	58	4.00	125/100
21	30/10	119	2,400	2	66	5.15, 6.30, 6.00 ..	141/87, 148/100, 174/114..
22	13/10	120	2,830	2	88	6.30, 5.15	200/114, 114/71
23	15/10	123	2,580	2	113	6.00	200/100
24	18/10	122	2,580	2	67	6.00, 6.45	210/108, 148/100
25	18/10	131	2,925	2	90	6.00, 2.15	190/91, 222/174
26	1/11	143	2,400	2	>101	4.00, 7.00	143/90, 182/71
27	2/11	128	2,340	2	90	5.00, 3.30, 5.00 ..	133/67, 160/83, 125?.....
						6.00, 4.30, 3.00 ..	114/59, 67/50, 83/67.....

treatment lasted 8 hours, the shortest $2\frac{1}{4}$ hours. The reason for the interruption of the dialysis after so short a time has as a rule been some complication or other or some technical mishap.

The effect of dialysis on the blood-N.P.N. As was expected, the effectiveness is greatest on high initial values and protracted dialysis. Less yield can be explained by for some reason or other lessened flow of blood through the apparatus.

In fig. 4, 12 exemplary experiments are recorded to allow a survey, showing the effect of dialytic treatment on the blood-N.P.N. We find that most of the animals die at proportionately low N.P.N.

1.

Nr.	N.P.N. in dialysate, g	Heparin			Hemorrhages				Bloodtransfusions, total ml	Cause of death			
		Total mg	Hours	Mg per hour/kg	Wound	Intestinal	Retroperti- toneal	Other hem.		Hem. colitis	Hem.	Pulmonary	Other causes
1	1.9, 2.6	140	25	2.4	—	—	—	+	26	+	—	—	—
2	1.3, 0.6, 0.4, 1.0, 0.7, 1.6, 0.8	507	132	1.6	+	—	—	—	170	+	—	+	—
3	1.1	110	23	2.2	+	—	—	—	23	+	—	—	—
4	0.6	73	17	1.6	+	—	—	—	30	+	—	—	—
5	1.6, 1.2, 0.8	158	48	1.1	+	—	—	—	35	+	—	—	+
6	0.4	28	3.5	2.3	—	—	—	—	0	+	—	+	—
7	1.4	95	17	2.1	—	—	+	—	16	+	—	—	—
8	2.1, 1.8, 1.4, 1.1, 0.5	443	100	1.2	(+)	—	—	—	100	—	—	—	—
9	1.6	80	19	1.5	—	—	+	—	17	+	+	—	—
10	0.8, 0.8	165	43	1.4	—	—	—	—	15	—	—	+	—
11	1.6	78	24	1.3	—	+	—	—	35	+	+	—	—
12	2.0, 1.3, 1.5	186	63	1.1	—	+	—	—	75	—	+	—	—
13	2.2, 2.2, 1.5	170	53	1.2	—	+	—	+	105	+	+	—	—
14	1.4, 1.7	120	32	1.2	—	+	—	—	42	+	+	—	—
15	1.4	60	15	1.5	—	—	—	—	15	—	—	+	—
16	0.8, 1.1 0.8	165	52	1.2	—	—	—	—	46	—	—	+	—
17	1.1, 0.5	120	38	1.2	(+)	—	—	—	60	—	—	+	—
18	0.4	35	4	1.3	—	—	—	—	0	—	—	+	—
19	0.9, 0.8, 1.5, 2.0, 1.2	282	97	1.0	—	+	—	+	152	+	+	—	—
20	1.5	68	16	1.8	—	+	—	—	55	—	+	—	—
21	0.9	55	18	1.3	—	—	—	—	15	—	—	—	+
22	1.0, 1.1	100	28	1.3	—	—	+	+	60	+	—	—	—
23	1.5, 1.3	?	?	?	—	—	+	+	47	+	—	+	—
24	1.5, 0.6	78	27	1.2	—	+	—	—	30	+	+	—	—
25	0.7, 1.5	120	42	1.0	—	—	—	—	40	—	—	+	—
26	1.0, 0.8?	91	55?	0.7?	—	—	—	—	32	—	—	—	—
27	0.8, 0.8	114	51	1.0	—	—	—	—	39	—	—	—	—

levels or that the N.P.N. was comparatively low when the last treatment was finished.

The following are complementary details: a) In 21 cases the blood-N.P.N. was determined after death. In one case the value was between 251—300 mg%, in four cases between 201—250 mg%, in six cases 151—200, in four cases 101—150, and in six cases below 100 mg%; b) in one case N.P.N. 12 hours before death was 125 mg%, in one case 13 hours before 87 mg%, and in a third case 18 hours before 118 mg%.

It is thus evident that the dialytic treatment has largely prevented

an increase in the blood-N.P.N., and that the animals in this group generally died from causes other than uremia. As a rule 4—7 hours' dialysis is sufficient to reduce the uremia to a moderate degree.

The amount of N.P.N. removed by dialysis depends on the blood-N.P.N. level, the duration of the treatment and the rate of the blood through the apparatus. The eliminated amount corresponds roughly to that which can be calculated from the following premises: a) The level of the blood-N.P.N. and its reduction during

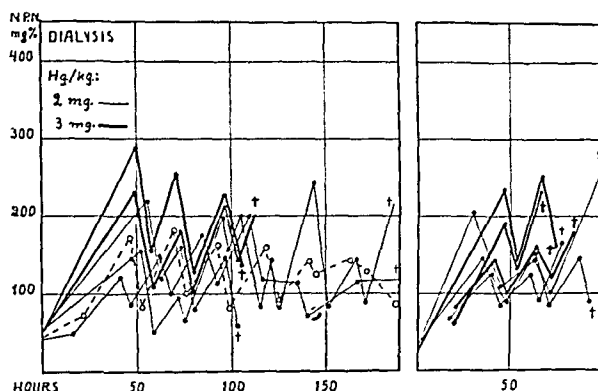


Fig. 4.
(Small circles: Survivors.)

dialysis, b) a uniform distribution of the N.P.N. in the body fluid of the animal.

The heparin dosage is reported as the total amount given to the animal during the recorded number of hours. Consequently the dialytic treatment as well as the arterio-venous anastomosis maintained between the treatments fall within that space of time. Further the amount of heparin, worked out per hour and kilo body-weight is reported. On the whole the development has tended towards smaller doses of heparin because of the consequent complication of hemorrhage.

The amount of transfused blood, i. e. the amount of blood given above the compensation for samples of blood has varied in different experiments, with varying hemorrhages. On the whole the amounts of blood are fairly small if the duration of the treatment is taken into consideration.

Under the heading *hemorrhages and causes of death* we report complications that have occurred, and have sometimes been to a smaller or greater degree the cause of death.

Hemorrhage complication, no doubt, essentially due to the he-

parinization, is the most common cause of death; hemorrhages have occurred from a) the operation wound through which the cannulae were inserted; as reported in an earlier paper on the technique in experiments on animals, profuse hemorrhages often occurred, which we only gradually learnt to control by an improved operation technique. This, consequently, is a complication without correspondence in the control animals. b) *The intestinal canal*: in at least 4 of the dialyzed animals large hemorrhages were found in the intestine. Such a complication was not to be seen in the control material. c) *Retroperitoneal*: In no less than 4 cases large hemorrhages of this kind were seen, probably caused by ruptures when the heparinized animal was tied on the operation table; for nothing of the kind was seen in the control material. d) *Other hemorrhages*: In two cases hemorrhages after pricks and in one case from a wound that the animal had contracted on its foot.

Thus the heparinization with accompanying tendency to hemorrhages has been the cause of death in more than half the cases of these mercuric chloride poisoned animals. The risk of this complication has been reduced but not eliminated by reducing the heparin doses, even to the risk of coagulation. Whether a better therapeutic result could have been achieved by fewer dialytic treatments and shorter time of heparinization will not be discussed here.

The tendency to hemorrhage appears to be greater with prolonged heparinizing. This is perhaps the explanation of the fact that serious hemorrhages have fortunately been rare in our clinical material, to be reported in a later paper.

Of other causes of death of these animals, we will here only mention that 2 died of pneumonia and 2 of probable emboli; in the latter cases, death ensued after rinsing the cannulae inserted in the blood vessels, in which coagula had formed owing to insufficient doses of heparin.

Summary.

Some experiences of experimental studies of dialytic treatment of uremia, caused by mercurial poisoning of rabbits are reported. The results, which are discussed in detail, may be summed up as follows:

Dialytic treatment has on the whole efficiently prevented increasing accumulation of N.P.N. in the blood. The heparinizing,

however, with its tendency to cause hemorrhages has under our experimental conditions to great extent caused fatal complications. By reducing the heparin dosage even to the risk of coagulation, the danger of this complication has, perhaps, been reduced, but not eliminated. The risk of hemorrhage appears to be greatest in prolonged heparinization.

Whether a better therapeutic result could have been achieved by fewer dialytic treatments and shorter time of heparinization will not be discussed here.

Literature.

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From the II. Medical Clinic of the University of Budapest.
(Director: Prof. E. Haynal, M. D.).

On the Diffusion of Penicillin.

By

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The physical properties of penicillin are determined theoretically by two facts viz. that its recognized species are water-soluble combinations of a molecular size not too large and that there are neither in vitro nor in vivo signs indicating a combination with either human or bacterial proteins. Its diffusion and excretion through the semipermeable membranes of the organism should thus have been expected to be similar to a crystalline substance. Actually this is not so. It has been proved first perhaps in its clinical use in meningitis with what difficulty it passes through the blood-liquor barrier. Rammelkamp and Keefer were able to point out that penicillin was not equally distributed in erythrocytes and plasma, its concentration being in the former only 10 % of the latter. Struble and Bellows examined systematically the distribution of penicillin and found that in the case of giving it intramuscularly the different tissues of the body were containing it simultaneously in the following order: kidneys, small intestines, lungs, mucous membranes of the mouth, bile, skin, liver, suprarenal glands, pancreas, heart, muscles, spleen. There was none in nervous tissues and bone marrow. These conditions of distribution cannot be explained by the measure of blood-supply of the different organs. The leading position of secretive organs is perhaps explained, although the importance of the bile should be greater in this respect than that of the lungs. Striking is the relatively disadvantageous position of the heart within the succession of susceptibility.

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Miller and Foster, examining diffusion of penicillin into the cavities of the body, stated that diffusion into the peritoneal cavity was easy and independent of its pathologic contents. It was less so into the pleural and synovial cavities. Penicillin passed through the placenta but only in half of its original concentration. Rammelkamp and Keefer say that tears do not contain penicillin but Struble and Bellows, 15 minutes after injecting 12,000 U. per kg body-weight, found a very high concentration (3.19 U.) in the tears of dogs, whereas there was hardly a trace to be found in the aqueous humour and cornea. Andrews, after a subconjunctival injection of 50,000 units of penicillin, could not find any penicillin either in the lens or in the vitreous body, while at the same time (20 minutes after injection) concentration in the cornea was 182 U., in the serum 3.5 U. It is remarkable that proportions in the other eye were just the same, though, at a lesser concentration. As can be seen, distribution of penicillin in the organism, almost independently of the manner of application, varies to a great extent.

Neither is the remaining quantity of penicillin in the tissues the same. According to Struble and Bellows penicillin could be detected two hours after injection in the bile only. According to Florey, Turton and Duthie penicillin in vivo can be found for a long time in secretions of wounds, even 48 hours after the injection of 100,000 U.

With regard to these conditions of resorption, distribution and excretion of penicillin we thought it necessary to make some researches on its diffusion-qualities. Also clinical evidences led us to do so. There were autopsy-results showing that penicillin, dispensed in due course and in sufficient quantity to sufficiently influence the clinical status, was not enough to exterminate bacteria in the lower levels of vegetations located on the endocardium which in vitro are sensible towards penicillin. This opinion is confirmed by Priest, Smith and Mc. Gee's paper. Another reason for starting on researches was that in our former experiments intending to prevent the excretion of penicillin, we succeeded in doing so by prescribing a diet rich in vegetable proteins, *e. g.* oat-flakes: this increases the synthesis of hippuric acid in the organism. (Mosonyi and others.) A high serum level with definite bacteriostatic power can be maintained similarly by giving 0.30 g aminopyrin *per os* to the examined subjects. (Mosonyi and Ducks.) We wanted therefore to be assured whether these proceedings were

without obnoxious influence on the other hand, viz. did not prevent the direct contact of penicillin and bacteria.

The ability of penicillin to penetrate an ultrafilter has already been proved by Petrányi and Rusznyák. Our aim was now to make experiments in conditions similar to those in living organism. A membrane formed by the combination of solutions of pure thrombin and crystallized fibrinogen, made by Laki and Gerendás, seemed to be most suitable for the researches of diffusional properties of penicillin. This proved to be suitable concerning its chemical qualities, as well as to measurability and sterility. The setting of our experiments — rather model-experiments — was done by agar discs and cylinders. An agar cylinder of 2 mm height and 10 mm diameter was placed on a Heatley agar-plate prepared as usual and above this 0.5, 1 and 2 units of penicillin in a glass-cylinder. After a pause of one or two hours at a temperature of 37° C the penicillin was sucked off with the help of a Pasteur pipette but the agar-plate was left in the thermostate. Comparing the diameters of the growing-inhibition of these, and those left to penicillin-influence for 24 hours, we found that there was no important difference. This proved that the whole effective quantity of penicillin had been concentrated in the agar-cylinder in the first hour already, or that after a certain degree of saturation of this agar-cylinder no further quantity of penicillin could pass through this filter. It seemed probable after this experiment, that penicillin had been adsorbed by agar-colloid and that the well-known rules of adsorption were valid in this process.

For the second experiment glass tubes of 2 mm diameter and 8 cm length were filled with agar solution and after congelation at room temperature 500 and 1000 U. of penicillin were overlaid. After having been kept for 24 hours at 37° C the cylinders were pushed out of the tubes and taking care of sterility, we cut them off into discs at exactly measured distances from the upper end. These discs were put on agar-plates infected by a 24 hours' bouillon culture of staphylococcus aureus Oxford. Dimensions of the inhibition-zones after 24 hours showed that, except for the first discs, the inhibition-zone of which was 30 mm in breadth, the inhibition-zones of the discs placed at the distance of 8, 14, 21 and 29 mm were almost equal (15, 14, 16 respectively). For the 1000 units-tube the inhibition-zones at 0, 8, 17 and 25 mm distances were respectively of 34, 20, 22 and 18 mm in diameter. The larger inhibition zone of the first disc may be caused by the penicillin

solution above it that cannot be removed completely. Sections of agar-cylinders overlayed by lesser quantities of penicillin (*e. g.* 10 or 50 O. U.) showed similar distribution.

Table 1.

Distance from the end of the agar-cylinder	Diameter of the inhibition-zone after overlaying	
	500 U.	1000 U.
0 mm	30 mm	34 mm
8 »	15 »	20 »
14 »	14 »	
17 »		22 »
21 »	14 »	
25 »		18 »
29 »	16 »	
	(mean values)	

Again another case was when agar mixed up with staphylococcus bouillon was poured into a test tube and after congelation overlaid by 4000 U. penicillin. 24 hours later there was a sharp limit of penicillin-effect to be seen at a depth of 20 mm beneath whereof turbidity demonstrated bacterial growth.

These model-tests convinced us of the fact that diffusing penicillin is evenly adsorbed by colloidal substances, without losing its efficiency and in such an adsorbed state, at a temperature of 37° C, it keeps its effect much longer than in watery solution.

The experiments as described above having been completed a Heatley glass was placed upon an infected agar-plate and some fibrinogen-thrombin solution was dripped into it. After congelation a solution of penicillin was laid over it. The fibrinogen solution was one of 10 ‰ the pH was set at 7.5; and corresponding to normal conditions of human organism, the penicillin solution used in this test was of lesser concentration. A fibrin-layer of 1 mm thickness having been prepared and exposed to the action of 0.5 U. penicillin for two hours, gave an inhibition-zone of 28 mm in diameter. Leaving the penicillin solution to act for 24 hours, the inhibition-zone did not grow, but remained essentially the same, being 26 mm. Using a fibrin-layer of 2 mm the corresponding data were 30, 28 and 32 mm. Thus the fibrin-layer shows the same qualities as the agar-cylinder does in the model-tests. If no penicillin was put over the fibrin, no inhibition could be detected: this was a proof for the zones above being caused by the diffused or adsorbed penicillin. (Table 1.)

Raising the quantity of penicillin (to 5 and 10 U.) a large inhibition-zone was the result, regardless of the thickness of the fibrin-layer. This means that even a fibrin-layer of 3--4 mm is not able to prevent diffusion of highly concentrated penicillin.



On the other hand no effect of 0.5 to 1 U. penicillin could be seen below the fibrin-layer of 3 mm.

The concentration of penicillin reaches, even in case of intravenous use, 0.3 to 0.1 units in blood for a very short time only. Thus it seems to be explained why bacteria at the depth of vegetations, often surpassing a thickness of 4 and 5 mm, are to be found in full virulence despite administration of penicillin.

In some of our cases, even a fibrin-layer of 2 mm, prevented diffusion of penicillin, whereas a layer of 3 mm did so in every case.

Trying to explain the principle of our observations, we added

to the penicillin solution substances furthering diffusion. To this purpose a 5 % urea solution and a 20 % solution of sodium dehydrocholicum was used. 0.5 U. penicillin could not penetrate through a 3 mm fibrin-layer (fig. A/1) not even by adding 0.05 ml of urea solution (Fig. A/2). The mixture of penicillin and sodium dehydrocholicum resulted an inhibition-zone of 14 mm under the same circumstances (Fig. A/3). (First it was ascertained, that neither urea nor sodium dehydrocholicum caused inhibition by themselves.) Using an urea solution 1 U. of penicillin gave a 17 mm wide inhibition-zone, while adding a drop of sodium dehydrocholicum the same quantity gave one of 18 mm. It was not possible to get penicillin-effect through a 2 mm layer with 0.5 U. and urea, whereas with sodium dehydrocholicum there was a zone of 18 mm. The urea solution has a pore-dilating effect, on the other hand the sodium dehydrocholicum solution acts on the surface tension and thereby seems to be a factor influencing adsorption.

Table 2.

Thickness of fibrin-layer	Inhibition-zone after overlaying 0.5 O. U. penicillin		After adding 0.05 ml of urea-solution Na. dehydrocholic.	
	during 2	24 hours	5 %	20 %
1 mm	28 mm	26 mm		
2 »	30 »	30 »		
3 »	—	—	—	14 mm
	overlaid by 1 U.			
3 »	—	—	17 mm	18 »

In order to elucidate the mechanism of this phenomenal facts 0.25 U. penicillin were added to 0.5 ml fibrinogen solution (before precipitation) and then thrombin was added. The precipitation of fibrin having taken place, the liquid pressed out of it was tested for penicillin but no traces of penicillin were found (Fig. A/4). Thus it must be presumed that fibrin adsorbs, but not filters penicillin. Increasing the quantity of penicillin it was found that 0.5 ml of a precipitated 10 ‰ solution of fibrinogen did not adsorb 1 U. of penicillin (Fig. A/6). In order to make fibrinogen adsorb 0.5 U. penicillin, the former must be precipitated by thrombin, since penicillin is to found unchanged in unprecipitated fibrinogen (Fig. A/5). The effect of sodium dehydrocholicum was found to be unchanged together with a 5 % aminopyrin solution which has, according to Eppinger and his collaborators a pore-blocking effect and the inhibition-zone remained, through a fibrin-

layer of 2 mm, again 26 mm. This fact supports also the presumption that penicillin is adsorbed by and not filtered through fibrin. As a direct proof for this presumption stands furthermore the fact that after having added sodium dehydrocholicum and penicillin to unprecipitated fibrinogen, the precipitate resulting from the addition of thrombin was of an unusual gelatinous nature and 0.1 ml fluid pressed out of it with difficulty, gave an inhibition zone of 24 mm. Finally having 0.5 U. penicillin adsorbed by fibrin, the precipitate was washed out repeatedly with physiologic salt solution and afterwards eluted by sodium dehydrocholicum, 0.1 ml of this fluid effected an inhibition-zone of 28 mm. Thus it can be said, that adsorption of penicillin can be prevented by diminution of the surface of the colloid system and even the adsorbed part of it can be regained in unaltered form (Fig. A/7).

Table 3.

	Diameter of inhibition-zone of 0.1 ml of the expressed fluid
0.5 ml fibrinogen sol. 10 % + 0.05 ml thrombin + 0.25 U. penicillin	—
0.5 ml fibrinogen + 0.5 U. penicillin	30 mm
0.5 ml fibrinogen + 0.05 ml thrombin + 0.5 U. penicillin	—
0.5 ml fibrinogen + 0.1 ml Na. dehydrochol. 20 % + 0.5 U. penicillin + 0.05 ml thrombin	24 mm
0.5 ml fibrinogen + 0.5 U. penicillin + 0.05 ml thrombin	—
The precipitate washed out with phys. saline sol., then eluted by 2 ml. Na. dehydrochol. 20 %	28 mm (mean values)

Table 4.

Thickness of fibrin-layer	Overlayered by	Diameter of inhibition zone
3 mm	0.5 U. penicillin	—
3 ,	0.5 ml U. penicillin + 0.1 ml Na. dehydrochol. 20 %	26 mm
3 ,	0.5 U. penicillin + 0.1 ml Na. dehydrochol. 20 % + 0.05 ml aminopyrin sol. 5 %	

The experiments, the intention of which was to state the increase of the diffusion-potential of penicillin in living organism by dispensing it together with sodium dehydrocholicum, remained without any result. The reason of this may be that plasma-concentration of penicillin injected shortly diminished below 0.25 units. This concentration does not seem to be high enough to

penetrate even through a fibrin-layer of 1 mm. It was technically impossible to prepare a thinner layer than that.

Summary.

1) Diffusion qualities of penicillin are different from those of other crystalline substances.

2) Penicillin in vitro, passing through jellies, is adsorbed by colloidal substances and its diffusion is greatly influenced by this adsorption.

3) Such an adsorption can be lessened by sodium dehydrocholicum and the penicillin once adsorbed can be newly eluted. Penicillin thus regained does not lose its former efficiency.

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Studies on the Circulation in Man.

I.

Technique of Venous Catheterization With Determination of Cardiac Output and Simultaneous Recordings of the Blood Pressures, the Electrocardiogram, Phonocardiogram and Respiration.¹

By

HENRIK LAGERLÖF and LARS WERKÖ.

(Submitted for publication March 13, 1948.)

The development of new techniques has opened new opportunities for investigation of the circulation in man. Studies now in progress at St. Erik's Hospital are mainly concerned with the work or failure of the heart in hypertension and organic heart disease. This paper reports the technique and equipment used.

Catheterization of the heart. The technique originally described by Cournand is used (1). The procedure has recently been discussed in detail by one of us (2). In this study a few modifications have been made. For local anesthesia Xylocaine (Astra) is used. It has the advantage over Novocaine that the effect lasts three to four times longer.

In the cases where simultaneous pressure tracings from more than one heart chamber is desired, two catheters are introduced, as the double lumen catheter (3) is difficult to place at will. One of the catheters is passed through the basilic vein and the other through the cephalic vein of the same arm. At first it was tried to put both catheters through the same vein. The vein was distended and there was a fairly large bleeding from the space be-

¹ Aided by grants from the State Medical Research Council.

tween the catheters. The blood between the catheters also showed a tendency to clot. When two veins are used no such complication occurs. Usually no difficulty is encountered in placing the tips of both catheters at will, in the pulmonary artery, right ventricle or auricle. Movement of one of the catheters does not influence the position of the other, which is advantageous.

Determination of the cardiac output. The cardiac output is determined according to the Fick principle as earlier described (2, 4). When several determinations are made with short intervals, the oxygen consumption is only determined on the same time as the first collecting of mixed venous and arterial blood. The oxygen consumption during the intermediate determinations is assumed to be the same as the mean value of the first and last determinations.

Recordings of pressures. The pressures tracings are obtained by connecting capacitive manometers according to Tybjaerg-Hansen and Warburg to intracardiac catheters or to arterial needles according to Cournand or Tybjaerg-Hansen (5—7). The natural frequency of the manometer system is tested according to Tybjaerg-Hansen and Warburg. Attached to arterial needles it was about 100 per sec. and with the catheters 20—30 cycles per sec. It is not significantly altered by different calibres of the catheters because of the stiff membranes of the manometers.

The damping of the manometers constructed for arterial pressure, high pressure manometers in the original work of Tybjaerg-Hansen, was accomplished by using needles for arterial puncture with an inside diameter of 0.2 mm, the damping of the manometers for normal pressure in the right heart, low pressure manometers, by the use of needles of about the same caliber tightly fastened inside the manometer. The damping of the manometer systems with arterial needle or catheter attached was calculated by Tybjaerg-Hansen to be critical. Thus the amplitudes should be correct up to a frequency equal to the natural, and then progressively falling. The phase lag in seconds should be equal for all frequencies, about 0.01 sec.

As we usually use arterial needles according to Cournand a short damping needle of 0.2 mm diameter was soldered into a conical adapter, which fitted into this needle. The adapter was tightly screwed to the manometer. In testing the natural frequency the damping was found in some attachments to be critical, in others slightly less. This does not invalidate the arterial tracing

because of the high natural frequency. In cases with high pressure in the heart a high pressure manometer was attached to the catheter.

The manometers are connected by means of three way stopcocks and rubber tubing to a pressure bottle containing 700 ml saline with 75 mg of heparine and 1 gram of sulfamethyl-thiodiazol. The whole system is freed from air by boiling. After boiling the manometers are stored with the stopcocks closed under water containing 1 gram chloramine per 1,000 ml until they are used. If stored so it has not been necessary to boil the manometers before every experiment.

It may happen that the damping needles in the low pressure manometers become partly plugged during the boiling and also when stored in water. This will result in overdamping. It is impossible to test the natural frequency before every use of the manometers to detect this error. It can, however, also be tested by controlling how many drops of saline the damping needle delivers per min. for a given pressure.

We have lately tried to overcome the difficulty of the changing lumen of the damping needle by using another damping principle. A small drop of a mixture of paraffine oil and refined petroleum is placed between the manometer membrane and the plane brass cylinder which together gives the capacity. As the dielectric constant of oils are greater than air this results in an increase of the sensitivity of the manometers to about the double. Thus the high pressure manometers can be used also for recording of low pressures. With a mixture of nine parts of paraffine oil and four parts of petroleum the damping of the high pressure manometers was about the same as with the damping needle. Precaution must be taken that the oil does not contain air bubbles.

The capacity of the manometer is part of one of two electrone coupled swinging electrical circuits, which are in resonance. One is connected to one grid, the other to another of a high frequency aggregate. Changes of the capacity results in a phase difference between potential and current in the connected circuit and hence in a potential difference between the two grids. The resulting change in anode current is amplified in a direct current amplifier, the output of which is connected to bifilar oscillographs, constructed by Elmquist. One iron nucleus torsion band oscillograph in an ordinary four lead electrocardiograph is exchanged by three such bifilar oscillographs. The deflection of the oscillographs is photographed

on a paper, moving with a fixed speed, on the same time as timer markings. The other three iron nucleus torsion band oscillographs are usually used for electrocardiogram, phonocardiogram and registration of the respiration.

For the maintenance of a constant zero point it is necessary to have constant potentials on the six volt lead accumulators, which maintains the catode current and also on the anode batteries of the high frequency aggregate and amplifier. The first of this conditions is obtained according to Tybjaerg-Hansen by loading the six volt accumulators during the recording with a current that is equal to the used. This is done by an alternating current rectifier, constructed by Philips. The anode potential of the amplifier of two channels is kept constant by a stabilized alternating current rectifier constructed by Elmquist and Wegner, that is connected to the main. In this way the drift of the zero point is insignificant during the course of one pressure recording. Standardization with a given pressure is, however, made before every tracing and the zero point controlled at its end. The low pressure manometers with their electrical connections were linear between pressures from 0 to about 70 mm Hg, the high pressure manometers between 0 and 350 mm Hg.

Respiration was registered by a pressure tracing from one nose opening or from an outlet in the valve used when collecting expired air or from the pressure variations in an modified Mareys pneumograph, fastened around the chest. These were connected with rubber tubings to a sensitive capacitive manometer according to Wegner or to a piezoelectric microphone for air pressure. The capacitive manometer was connected to a similar electrical system as the blood pressure manometers. Absolute pressure values can be obtained after standardization of the manometer. The piezoelectric microphone, constructed by Elmquist, is designed for small air pressures. The principles of the electric system is the same as for his arterial pressure microphone (8). The piezoelectric device has a time constant of about one second. Hence the curved obtained in this way merely gives the rapid phases of respiration but gives no true picture of the actual pressure of the respiratory air or of the pneumograph.

The phonocardiogram is recorded with a piezoelectric microphone according to Elmquist. It was connectd to the fourth lead of the electrocardiograph, which had filters for phonocardiography.

External pulse curves have been recorded by connecting piezo-

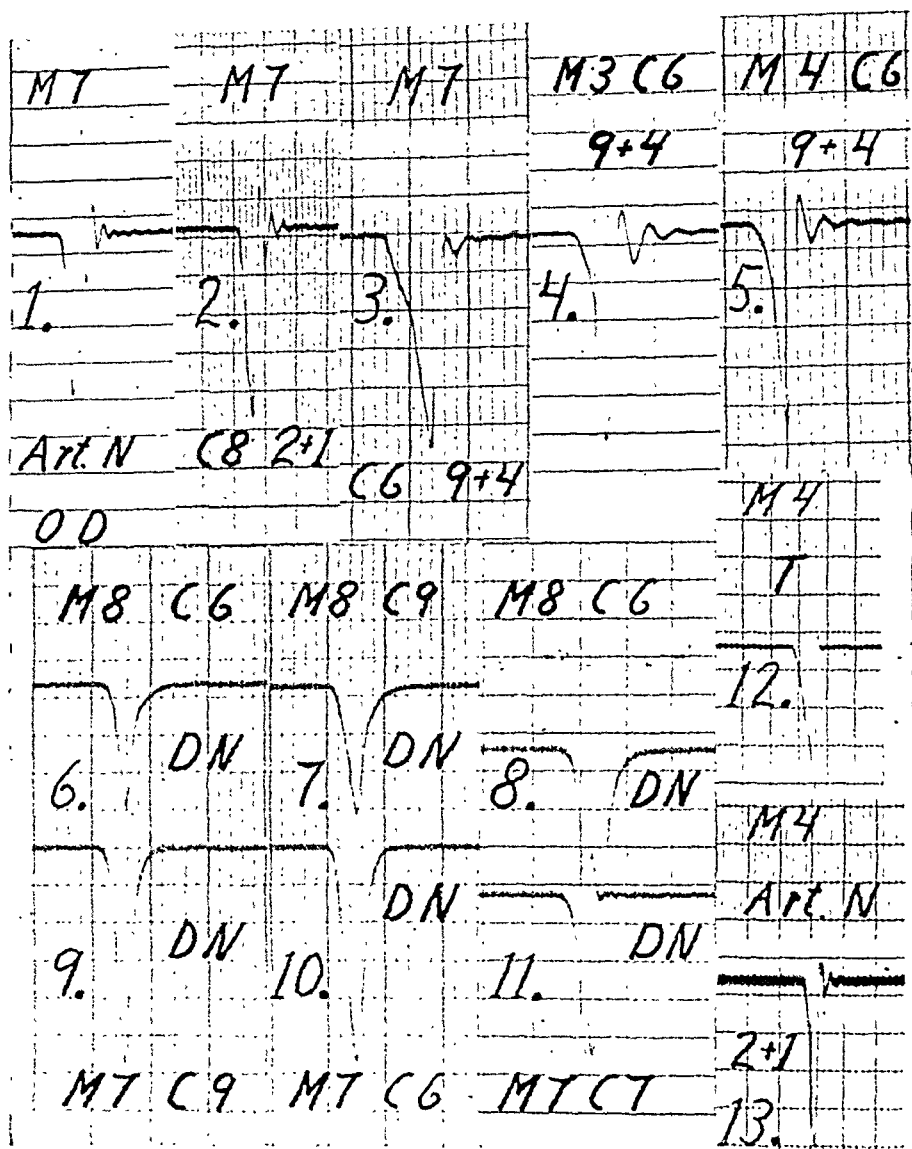


Fig. 1. Abbreviations: M 3, M 4 = high pressure manometers 3 and 4.
 M 7, M 8 = low pressure manometers 7 and 8.
 C 6—9 = catheters 6—9 F.
 Art. N = arterial needle according to Cournand.
 Art. T = arterial needle according to Tybjaerg-Hansen, and Warburg.
 2 + 1 and 9 + 4 = proportion between paraffine oil and refined petroleum in the capacity.
 DN = damping needle according to Tybjaerg-Hansen and Warburg.
 OD = no damping.

1. Frequency 40, manometer undamped. 2. Frequency 25, manometer slightly underdamped. 3. Frequency 23, manometer slightly overdamped. Coupled oscillations. 4. Frequency 23, adequate damping. 5. D.o. 6—10. Manometers slightly to considerably overdamped. 11. Frequency 22, adequate damping. 12. Frequency about 40, adequate damping, (probably air in the manometer). 13. Frequency about 40, adequate damping, (probably air in the manometer).

electric pulse microphones to one or two of the leads of the electrocardiograph according to Elmquist and Porjé (8).

Tests for parallax, frequency, damping and time lag between mechanical and electrical impulses.

Fig. 1 shows tests for frequency and damping with our manometer under different conditions. For analyse of the results we refer to the original works of Tybjaerg-Hansen and Warburg.

The parallax was tested by connecting the oscillographs parallelly in an electrical circuit of one millivolt. The deflection was recorded when the circuit was established. The parallax was less than 0.1 mm. With the usual speed of the paper of 4 cm/sec. this is less than 0.0022 of a second (fig. 2 A).

The difference of transmission time of electrical impulse as recorded with the EKG and simultaneous mechanical impulses from the tips of the catheters filled with water and connected with our low pressure manometer was tested in the following manner. The tips of the catheters, filled with air-free saline and connected to the low pressure manometers, were inserted into holes in a rubber stopper, which was attached to the open end of a syringe that was steadily mounted vertically. The plunger of the syringe was inserted from the other and connected with one pole of a 4 volt battery and with one of the electrocardiograph electrodes. The other pole was connected with the other electrode which was held in the hand. Tapping the plunger with one finger initiated simultaneously electrical and mechanical impulses, that were transmitted to the oscillographs of the EKG and of the manometer. The resulting deflection were photographed using a camera speed of 10 cm per sec. Fig. 2 B illustrates that the time interval between the beginning of the electrical and mechanical deflections was approximately 0.01 sec. Cournand et al. have reported the same time lag between string galvanometers and Hamilton manometers (9).

Measurements on the tracings. The zero-point for cardiac pressures were the same as used by the Bellevues group, 5 cm below the angle of Louis (10). The same reference point was used for other pressures.

Tracings were routinely measured for pressure values in every beat during a normal complete respiratory cycle and the result averaged. Mean pressures were determined by planimetric integration or by drawing the curve on transparent millimeter paper and comparing the weight of the cut curve with the weight of

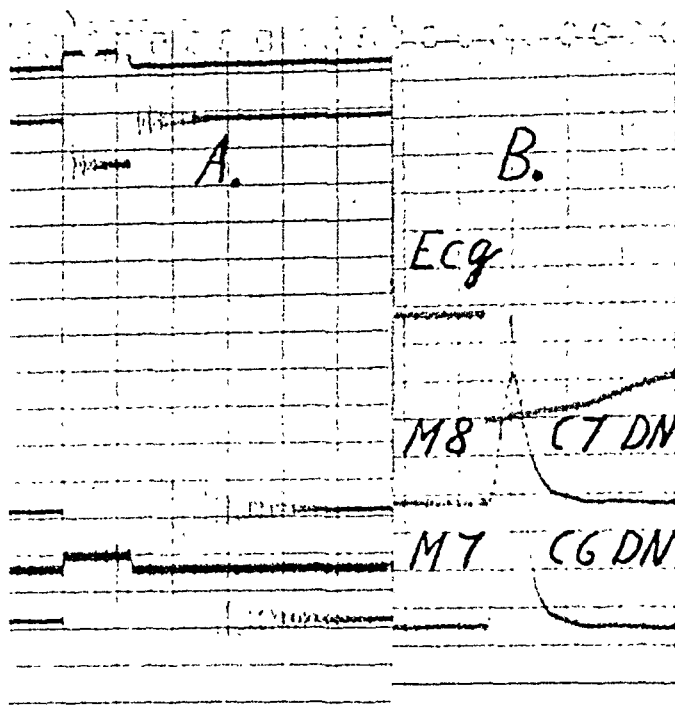


Fig. 2.

A. Test for parallax.

B. Test showing the difference in transmission time between electrical impulses from the ECG and mechanical through catheters. Abbreviations as in fig. 1.

standard pressure. By further cutting the mean values of different phases of the cardiac cycle could be obtained. In special cases, where the size of the curve was small, it was projected to a greater scale and the ordinates for every 0.02 sec. were measured. The arterial measurements include average maximum (systolic) average minimum (diastolic) and mean pressures. For the auricles usually only the mean pressure was measured.

The systolic ventricular pressure, VPS, in smooth curve was determined as the mean of the maximum pressures. Frequently the systolic part of the curve has an oscillatory character with the highest peak immediately following the isometric contraction. We attribute the oscillation to movements of the catheter and when they occur we chose the mean value of the peak and the following dip as the systolic pressure. The pressures at two moments of ventricular diastole were measured, at the lowest point following opening of the A—V valves and at the end of auricular systole or when this was not apparent in the ventricular tracings

just before the onset of the ventricular systolic rise. These points were designed, VPD_1 and VPD_2 , respectively. When oscillations due to catheter movements occurred the values for the peak and dip was averaged in the same way as in the systolic part of the curve. The difference between VPS and VPD_2 is designated as the pulse pressure PP.

The pressure in the pulmonary artery were measured in essentially the same way as the systemic arterial pressures. When oscillations occurred in the systolic or diastolic part the mean values between the peaks and dips were used.

Errors of Technique and Interpretation.

1. Reference to an incorrect Zero value.

The Bellevue group has pointed out that a horizontal plane 5 cm below the angle of Louis can differ from a zero plane exactly through the centre of the heart as much as 2 or 3 mm of mercury or even more with the phases of respiratory and cardiac cycles. Hence no value of pressure except pulse pressures has a final precision greater than ± 2 or 3 mm of mercury when patient to patient comparison is made. In a given patient the tip of the catheter may be situated in a region where the blood velocity is high and hence the pressure low and reversely. This should give pressure differences of as much as 3 mm of mercury during periods of rapid flow.

2. Technical errors due to the equipment.

In spite of all precautions small air bubbles may enter the manometer and decrease the natural frequency. The same result is obtained when the attachments do not fit exactly. Further damping needles may be partially plugged and overdamping result. Such errors are not always easy to detect from the form of the curve. They result in distorted amplitudes and increased time lag between the pressure and its registration. They invalidate the mean values only little.

The complicated electrical apparatus is vulnerable. The different contacts may loosen, the potentials of the batteries decrease etc. When such errors occur the apparatus usually does not response at all. In other cases the zero-point will be unstable or make sudden jumps, which are easy to detect.

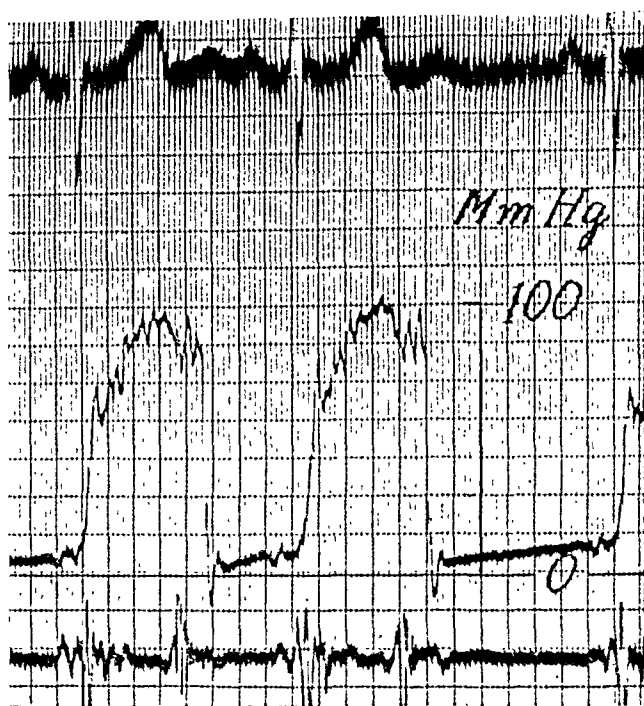


Fig. 3. Widened systolic part of the right ventricular pulse curve due to obstruction of the catheter after the closure of the pulmonic valves in a case of ventricular septal defect. The obstruction lasts between the main oscillations of the second tone and the third tone. — Note the following details: The auricular systole gives a biphasic pressure wave in the ventricle, starting with the negative part. The auricular sound starts synchronic with the positive part. The main oscillations of the first tone are synchronic with the isometric contraction, the following oscillations with the ejection phase. Small low frequency initial vibrations of the second sound starts already at the point where the pressure in the ventricle has reached its maximum. The main oscillations are synchronic with the incisure of the curve, which marks the closure of the pulmonic valves.

3. *Artefacts due to catheter motion and sudden obstruction at the end.*

The oscillatory character of the pulmonary and ventricular curve probably depend on catheter motions, because only small oscillations occur in tracings obtained by direct puncture during operation. The way to eliminate their influence on the calculated pressures is already discussed. Another common artefact is sudden obstruction of the catheter for a short period of time, most frequently seen in the ventricular tracings immediately after the closure of the pulmonary valves. It gives a false impression of the length of the ventricular systole. It is recognised by comparing the ventricular pulse curve with the pulse in the pulmonary artery or with the phonocardiogram (fig. 3).

Summary.

1. A technique for determination of cardiac output and obtaining simultaneous tracings of the blood pressures in one or more right heart chambers and the arterial blood pressure, the EKG, the phonocardiogram and the respiration is described.
2. Tests for natural frequency and damping of the manometers and parallax of the oscillographs are illustrated.
3. The principles in analyzing the pressure records are set forth and discussed.
4. Possible sources of error are discussed.

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Book Review.

A symposium on the use of isotopes in biology and medicine. 445 p. Price: \$ 5.00. The University of Wisconsin Press, Madison; 1948.

The use of isotopes in medicine is rapidly increasing. Still there is a general belief that we are only seeing the very first introductory experiments that will develop into everyday tests for most of our important metabolic disorders. What roentgenology has meant for the study of the hidden *structures* in the organism this new science will probably be able to give us regarding metabolic *functions*.

The Symposium was held in Madison in September 1947 and the chief reviewers include such prominent scientists as Urey, Seaborg and Nier on the purely physical side and Clarke, Bloch, Chaikoff, Hamilton and a number of other eminent workers in biology and medicine on the other.

The American concept that a limited number of people with common interests should meet for informal symposia with discussions on special topics seems very important and should be copied in Scandinavia. This volume is an excellent example of the wide scope of this field in biophysics and also a promise of future developments along many lines. Because of the large number of contributors it is obvious that the different parts vary somewhat in didactic and other qualities. On the other hand the volume gives a very thorough representation of the general meaning of recent work with isotopes and also of their international aspects and the future development of atomic energy.

The book may be recommended to all interested in biological and medical problems of the present and the future.

Jan Waldenström

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observations of Lövegren (1942). Under the same experimental conditions the latter found increases ranging from 46—102 mg per cent glucose in 12 normal individuals.

Geill regards an increase of 46 mg per cent, or above, as normal, which is in accordance with results obtained by Sucksdorff and Lövegren.

The Effect on Blood Ketone Bodies in Subjects with Normal Liver Function.

Numerous experiments have shown that adrenalin is capable of increasing the blood ketone concentration in normal subjects (*i. e.* subjects with normal liver function). Thus Hubbard & Wright (1921) in many cases found increased blood ketone concentrations following the administration of $\frac{1}{2}$ —1 mg adrenalin. Kugelmann (1931) found a considerable rise in blood ketone concentration one hour after a subcutaneous injection into normal subjects. Raab (1926) and Salomonsen (1930) found a considerable rise in blood ketone concentrations in starving dogs and human subjects respectively, concentrations which increased with the duration of the inanition period. In phlorrhizin-poisoned rats Anderson & Anderson (1927) were able to demonstrate a transitory rise in urinary excretion of ketone bodies following adrenalin injection. Evidence to the same effect was presented by Hirschhorn & Pollak (1927) in experiments upon starving phlorrhizin-poisoned rabbits. Beumer (1923) injected adrenalin into mammals 12 hours after the last meal and found ketonuria. Hirschhorn & Pollak (1927) found that in the adult the effect of adrenalin is most pronounced in the case of an already existing ketonuria, whereas no effect is to be observed if abundant quantities of carbohydrate are administered. In experiments on artificially perfused isolated livers Blixenkrone-Møller (1938) demonstrated that adrenalin, when added to the perfusion fluid, will bring about an increase in the concentration of ketone bodies therein.

The Effect on Blood Sugar in Hepatic Disease.

Barok & Rednik (1928) observed flat blood sugar curves in 14 patients with catarrhal jaundice, but in 6 patients with mechanical jaundice normal curves were observed following the administration of adrenalin, Kugelmann (1929) studied the blood sugar after

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From the Kommune Hospital, Medical Department VII, Copenhagen.
(Chief: Tage Bjerling, M. D.).

The Influence of Adrenalin on Blood Sugar and Blood Ketone Bodies in Normal Individuals and in Patients Suffering from Hepatic Disease.¹

By

AAGE WARMING-LARSEN.

Copenhagen.

(Submitted for publication February 6, 1948.)

Previous Investigations.

The Effect on Blood Sugar in normal Individuals.

It is generally known that adrenalin has a blood-sugar-raising effect in normal individuals. I shall not embark upon a survey of literature on this subject but only refer to Geill's work on the blood sugar curve following the administration of adrenalin to patients with hepatic disease. In this work, which appeared in 1943, previous investigations into the effect of adrenalin in normal individuals are subjected to a critical judgment. Geill emphasizes that adrenalin is most suitable for intramuscular injection. Subcutaneous injection results in irregular absorption of the adrenalin, depending on local factors. Intravenous injection is partly not without danger, partly it produces a smaller increase, presumably on account of destruction.

Following intramuscular injections of 0.14 mg adrenalin per kilogram of body weight (not exceeding 1 mg adrenalin, however) Sucksdorff (1930) found increases in normal persons ranging from 47—125 mg per cent glucose which is in accordance with the

¹ This investigation was performed with the support of Miss P. A. Brandt's Bequest.

observations of Lövegren (1942). Under the same experimental conditions the latter found increases ranging from 46—102 mg per cent glucose in 12 normal individuals.

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adrenalin injection. He introduced the term »The Hyperglycemic Index» which he defined as follows:

$$\frac{\text{Maximum Blood Sugar}}{\text{Minimum Blood Sugar}}$$

In normal individuals this ratio was found to be 1.5, or above, whereas it was notably lowered in diseases of the parenchyma of the liver.

Several authors — *e. g.* Sucksdorff (1930) and Brulé & Althausen (1931) — support the view that the adrenalin blood sugar curve is flatter in diseases of the parenchyma of the liver than in obstructive jaundice.

Furthermore a great Danish work appeared in 1943 written by Geill. The findings of the latter are as follows: After intramuscular injection of 1 mg adrenalin a rise of blood sugar over and above 46 mg per cent will indicate the presence of obstructive jaundice, whereas increases below this figure will indicate impairment of the parenchyma of the liver. The side-effects of adrenalin are pointed out and the author calls attention to the fact that they bear a strong resemblance to the symptoms of incipient hypoglycemia. Says Geill, the side-effects are no more pronounced than to permit of this test to be regarded as one of practical importance in the differential diagnosis of cholelithiasis from acute hepatitis.

Lövegren (1931) has examined 160 cases of different types of hepatic disease. This author considers the adrenalin test a great diagnostic aid, particularly when it is desired to differentiate functional jaundice from mechanical jaundice. It is not an infallible test, but according to Lövegren it compares reasonably well with other liver function tests employed.

The Effect on Blood Ketone Bodies in Hepatic Patients.

Brentano (1933) examined 2 patients with liver disease in whom the rise of blood sugar was remarkably small in response to adrenalin. Likewise he observed only a slight increase of blood ketone concentration. He explains this in the same way as did for instance Kugelman (1929) and maintains that the failing increase of blood sugar is due to a reduction in the amount of liver glycogen. On the basis of this erroneous inference he further concludes that a reduction in liver glycogen is not tantamount to increased formation of ketone bodies.

Seelig (1929) examined a number of liver patients and found only slight increases, if any, in blood ketone concentration after adrenalin injection. It seems to appear from the few investigations available that when the liver is affected only slight increases of blood ketone concentration are observed in response to the administration of adrenalin.

The Writer's Investigations.

Technique. The Hagedorn-Norman Jensen method was employed as usual for blood sugar determinations.

Jacob E. Poulsen's micro test (1941) was employed for determining ketone bodies. Tests for determining acetone + aceto-acetic acid and β -oxybutyric acid were, however, performed separately, which yields considerably more accurate results. The total amount of ketone bodies is quoted as hydroxybutyric acid.

Blood sugar and blood ketone concentrations were determined in 5 normal individuals (not affected with liver disease) with negative Takata-Ara reaction and normal icterus index. In addition I have examined 5 persons with epidemic hepatitis, all with an icterus index over and above 90.

Blood tests were performed at 15-minute intervals during a 3-hour period and following adrenalin injection (0.014 mg adrenalin per kilogram of body weight — not exceeding 1 mg, however). In view of the somewhat obscure conditions regarding the liver glycogen and its mobilization in hepatic disease the adrenalin tolerance test was performed twice on each patient, *i. e.* both before and after a starvation period lasting for three days (the post-starvation tests having thus been performed on the morning of the fourth day). In the course of a starvation period of the duration a normal individual will have mobilized his liver glycogen.

The five normal individuals and the five liver patients gave the same results within each group. I shall therefore confine myself to give one example only of each of the tolerance tests.

Fig. 1 shows the rise of blood sugar in a normal subject after adrenalin injection both before and after three days' starvation. Prior to the starvation period an increase from 70 mg per cent to 115 mg per cent is obtained, whereas an increase from only 61 mg per cent to 80 mg per cent is observed after the starvation. Consequently, the increase after starvation is smaller both absolutely and in relation to the initial level.

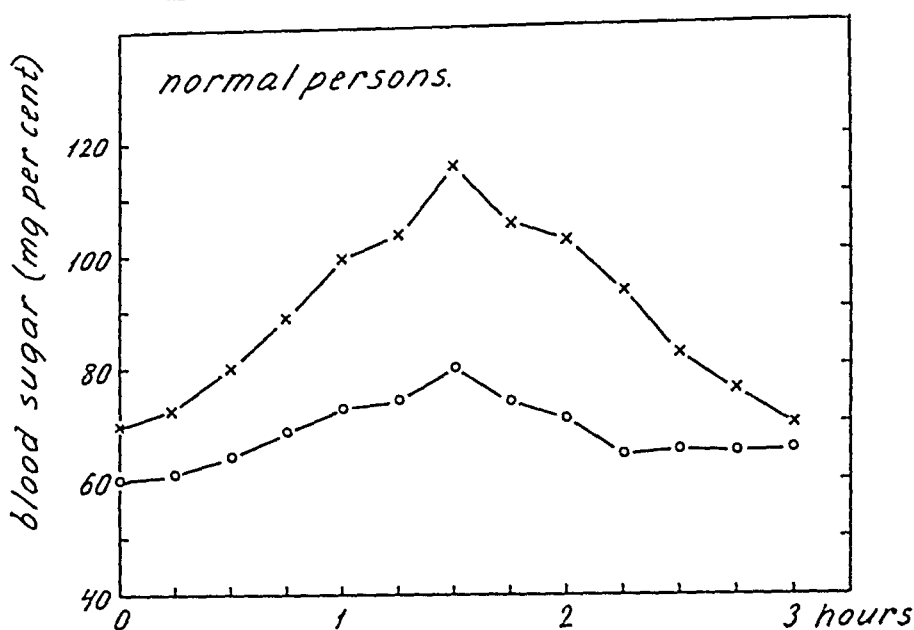


Fig. 1.

Fig. 2 shows corresponding experiments on a patient with acute hepatitis. The curve obtained before starvation is much flatter than normal. After starvation it has become even flatter, if possible, and, as in normal subjects, the initial level is somewhat lower.

The curves in fig. 3 show the course of blood ketone concentration in a normal subject after the injection of adrenalin both before and after 3 days' starvation. Before starvation no increase was observed following adrenalin injection. After the starvation period

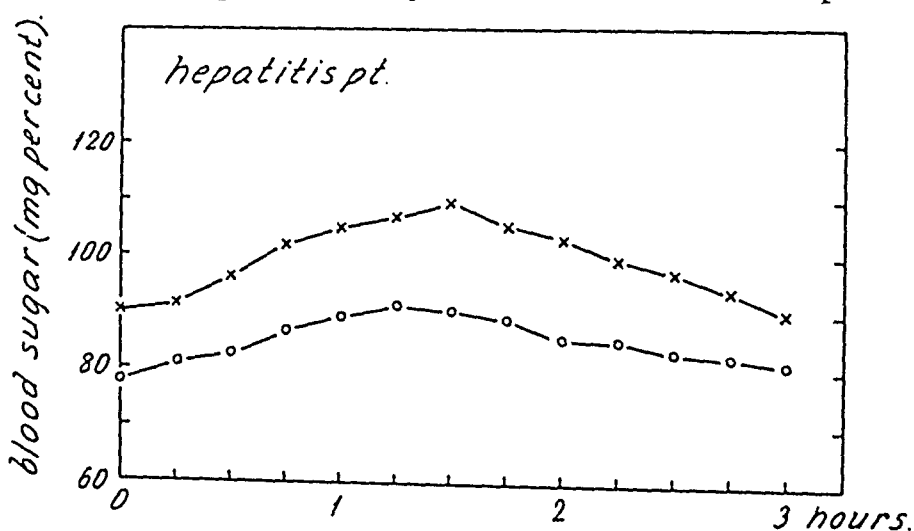


Fig. 2.

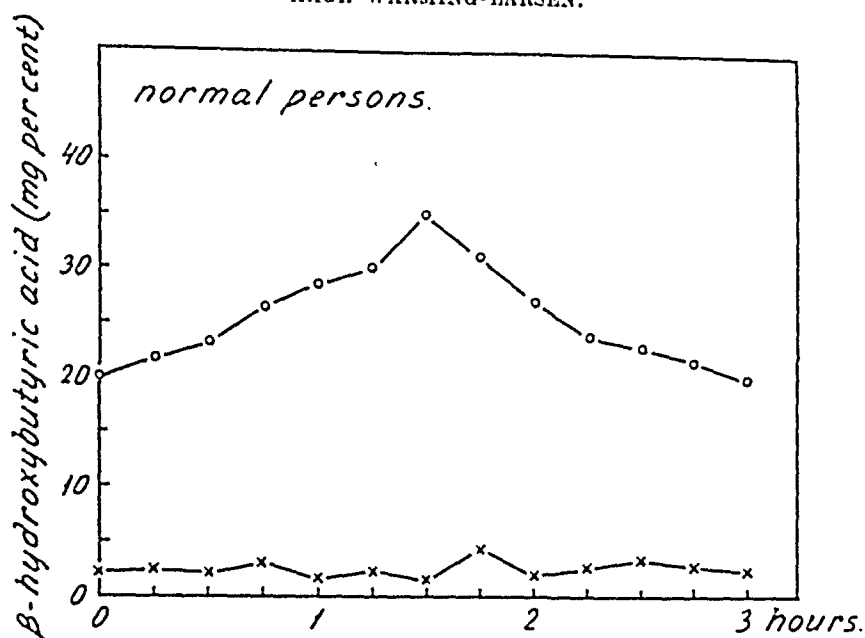


Fig. 3.

the initial level of blood ketone bodies is seen to be much higher and there is a considerable increase the peak of which as to time corresponds to the vertex of the bloodsugar curve in normal subjects following adrenalin injection.

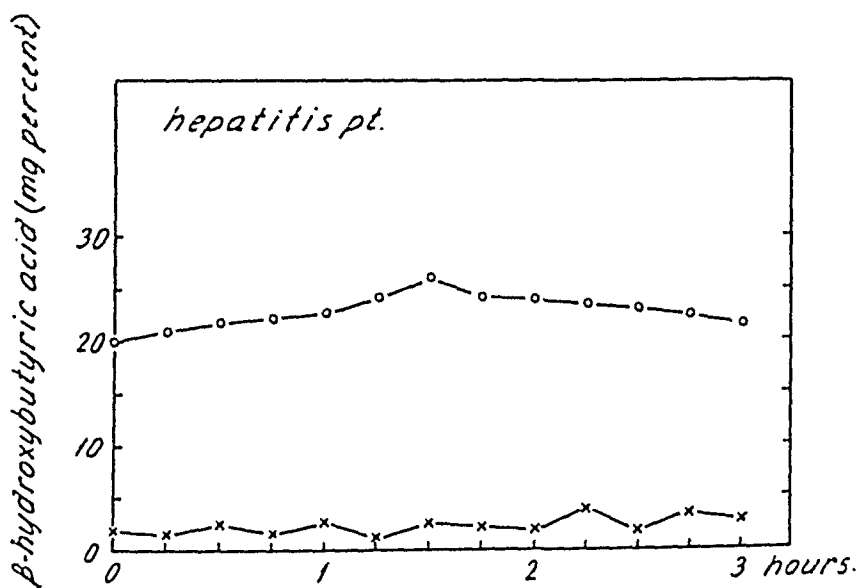


Fig. 4.

Fig. 4 shows corresponding experiments on a patient with acute hepatitis. Like in normal subjects there is only a slight amount of ketone bodies in the blood before starvation, and there is no rise in this concentration after adrenalin injection. It is remarkable that the blood ketone concentration after starvation is just as high as in normal subjects. Furthermore it is seen that adrenalin, when administered to hepatic patients after starvation, produces only a slight increase of ketonemia.

Discussion.

It is generally known that adrenalin has a blood-sugar-raising-effect owing to its ability to mobilize liver glycogen. Thus, Mann & Magath (1922) found that adrenalin had no effect on animals with an Eck's fistula or on animals which had been hepatectomized beforehand.

The significance of the muscle glycogen is much more difficult to explain. It is possible, as is well known, to deplete the store of glycogen in the liver, whereas starvation must be combined with muscular activity if it is desired to make also the muscles free from glycogen.

Adrenalin cannot mobilize the muscle glycogen in the sense that it is capable of transforming it into glucose which passes into the blood. On the other hand Cori & Cori (1928) are of opinion that adrenalin is capable of transforming muscle glycogen into lactic acid which through the blood is carried to the liver to be stored there in the form of glycogen, whereas a transport of liver glycogen to the muscles is thought to take place exclusively in the form of glucose. It should, however, be strongly emphasized that whereas the decomposition of liver glycogen into glucose takes place most rapidly under normal conditions, the muscles will only sluggishly and to a rather slight degree give off their glycogen to the blood in the form of lactic acid.

The fact that the blood sugar curves obtained after adrenalin injection are considerably flatter when the patient has been starving for three days beforehand is quite in keeping with the theory that the main effect of adrenalin in this connection is the mobilization of the liver glycogen since this has been practically eliminated after three days' starvation.

The essential increase of ketonemia which, on the other hand, is demonstrable in normal subjects after three days' starvation can

most likely be explained by the fact that the predominant metabolism of fats already taking place in the organism is activated further by the administration of adrenalin which has a stimulating effect on metabolism as a whole. The ketonemia which already exists in consequence of the starvation and is increased by the administration of adrenalin can surely be regarded as appropriate. The organism will transitorily increase the primary partial fat oxydation in the liver to such an extent that the blood ketone concentration will rise, and the increased supply will then facilitate the combustion of ketone bodies in the peripheral tissues.

It is difficult to give any direct explanation of the flat blood sugar curve obtained after adrenalin injection into patients with hepatic disease. In contradistinction to the opinion of previous investigators (*e. g.* Kugelman) Krarup (1939) proved that the amount of glycogen stored in the liver is normal in patients suffering from hepatitis. One might then think that the liver glycogen was mobilized with greater difficulty in such conditions, but this does not seem to be absolutely correct.

In connection with some investigations into ketone metabolism in hepatic disease which I made at Department III of the Kommune Hospital (Copenhagen), Poul Iversen, M. D., physician in chief, in a number of cases performed liver biopsy on patients with hepatitis at the close of a three day's starvation period (Warming-Larsen (1947)). The liver glycogen turned out to have practically disappeared. This means that in hepatitis the liver is perfectly capable of mobilizing glycogen. Consequently, it may be established that the enzymatic factor which transforms glycogen into glucose is intact in parenchymatous lesions of the liver. Hence the interpretation of the flat blood sugar curves obtained after the administration of adrenalin to hepatic patients will have to be modified as follows: *adrenalin is not capable of mobilizing liver glycogen in such patients, which is a different matter.* For some unknown reason adrenalin has no effect under such circumstances, a reason which presumably is connected with some condition in the diseased liver itself, but does not influence the general ability to mobilize glycogen.

As mentioned before the inanition ketonemia is just as pronounced in patients with hepatitis as in normal subjects. Adrenalin tolerance tests performed after starvation revealed no rise of ketonemia which is in contrast with results obtained in corresponding experiments on normal subjects.

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Spontaneous Pneumohemothorax.

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Introduction.

Since Rolleston and Pitt, in 1900, described the syndrome of the so-called spontaneous hemopneumothorax, reports have been published of about 50 cases. In his monograph on spontaneous pneumothorax in persons apparently healthy, in 1932, Kjærgaard mentions eleven cases, two of whom were his own and one a case published earlier by a Danish author (R. Kiær, 1923). In 1935 Frey collected 13 case histories from the literature. Of these only 10 could be said to be really spontaneous as 3 of them were already suffering from manifest pulmonary tuberculosis. A couple of years later Hopkins (1937) succeeded in collecting 43 cases from the literature published since 1900. Of these 43 eleven were tuberculous. Hopkins added 3 cases of his own, observed in the University Clinic in Philadelphia. Hartzell, in 1942, found 40 reports of non-tuberculous cases and added 3 of his own (+ a fourth one in an addendum) from the Cleveland City Hospital. The cases reported by Birch (1938), Baumont (1938) and Quinlan (1940) are not included in Hartzell's review. Since then Franklin (1945) has reported one case, but of course the war years have directed the interest towards other subjects.

The first description of the syndrome appears to have been made by Laënnec (1828) who found air under pressure and blood

in the pleural cavity at necropsy. The first case which was successfully treated by puncture appears to be the one reported by Whittaker in 1876. As in the case of all other diseases the frequency of this affection is without a doubt much higher than the published cases imply. In several instances a small effusion in spontaneous pneumothorax is not examined at all, as only the air is tapped off. In addition, several physicians do not report individual cases which do not afford new contributions towards elucidating the syndrome. Therefore, several cases have probably been observed without being published. Wilson's report of 2 cases thus brought to light 7 previously unpublished case histories. A few authors have observed several cases in their hospitals in the course of a short time, *e. g.* Korol (1936) 4 and Lour'a (1938) 5 at the Sea View Hospital.

Writer's Cases.

Below the writer will report 3 cases treated at the Clinic of Thoracic Surgery during the spring of 1946. None of them is quite typical of the disease. One of the cases (No. 1), however, only differs from the majority of those published hitherto in the onset of the symptoms. The patient was hospitalized for a perforated gastric ulcer and was thereupon treated for heart disease — by no means a rare variant of the syndrome and one that is important to know.

The other two patients to be reported below exhibited bilateral cystic lung. This underlying cause does not appear to have been observed in the cases published hitherto, at any rate not in this form. One of the two patients (No. 2) was a female (the second or third female reported in the literature) who is one of the very few patients older than 40. In the other case of cystic lung attended with pneumohemothorax (No. 3) the disease later had to be treated with an injection of a solution of silver nitrate, before the lung could re-expand. This form of therapy is well-known in cases of pathological pneumothorax, but it does not appear to have been used hitherto for pneumohemothorax and cystic lung, although it has been used for pulmonary cysts (Field & Rosenberg).

Case Histories.

Case No. 1. (Case rec. 116/46). Ship's cook, aged 30. Hitherto healthy. On March 26, 1946 an exertion was followed by sudden pain in the

chest and dyspnoea, thereupon abdominal pain and collapse. Immediately hospitalized for perforated gastric ulcer. Transferred to a department of medicine for heart disease. Received morphine and strophanthin. Hemoglobin percentage 58, erythrocyte count 2.55 millions. X-ray on March 28: Pneumothorax, collapsed lung, large effusion. The effusion was extremely hemorrhagic. As the fever persisted (38°—39° C.) and as the effusion went on re-collecting in spite of repeated thoracocenteses, the patient was transferred to the Clinic of Thoracic Surgery on April 29. On admission there was marked dyspnoea. Hemoglobin 96 per cent. X-ray: Large effusion, small pneumothorax. On April 30 3,150 ml of a hemorrhagic effusion with a hemoglobin percentage of 15 was tapped off the left pleural cavity and replaced by 1,500 ml of air. The temperature immediately fell to normal and the dyspnoea ceased. Three thoracocenteses during the following two weeks yielded a further 500 ml of effusion with a hemoglobin percentage of 10. At the same time a total of 1,050 ml of air was evacuated. On May 11 the lung had expanded completely. On May 25 the patient was discharged. The tuberculin reaction (up to 100 units) was negative. The pleural effusion failed to exhibit tubercle bacilli upon two cultivations. Wassermann reaction negative. Blood group O. Weight 74 kilos. Electrocardiogram normal. Blood pressure 130/75. Immediately after the discharge the patient went to Shanghai and has, therefore, not been followed up.

Summary of the Case: Following a severe exertion a male, aged 30, who hitherto had had healthy lungs and exhibited negative tuberculin reaction, got a pneumohemothorax with shock-like severe symptoms which first were interpreted as a perforated gastric ulcer and later as heart disease. The amount of the hemorrhage may be estimated at about 2.5 litres according to the body weight and the hemoglobin percentage (by the method of Aalkjær). The pulmonary fistula quickly closed spontaneously and the hemorrhage ceased. It was not, however, until the effusion had been thoroughly evacuated that the last remnants of clinical symptoms, in the form of dyspnoea and fever, disappeared simultaneously with the re-expansion of the lung. No underlying cause could be demonstrated.

Case No. 2. (Case rec. 131/46). A female, aged 42. Had always been short of breath. Eight years ago left-sided spontaneous pneumothorax with effusion (not analysed). For the last 3 years the patient had been under Tuberculosis Centre supervision, but no abnormal X-ray findings had been made. Negative tuberculin reaction. On May 12, 1946 — without previous exertion, cough etc. — sudden pain in the chest and dyspnoea. Three days later admitted to hospital where valvular pneumothorax was diagnosed. On May 16 a drain was inserted in the right anterior chest-wall. Apart from large quantities of air 250 ml of a hemor-

rhagic fluid were evacuated. On account of difficulties in preserving the drainage the patient was transferred to the Clinic of Thoracic Surgery on May 20. There was excessive dyspnoea and a miserable pulse. Hemoglobin 115 per cent. Treated with continuous evacuation of hemorrhagic pleural exudate and air, and oxygen tent. Although X-ray revealed the lung to be almost fully expanded the dyspnoea was exacerbated. Thoracoscopy on May 27: Pleura injected and with fibrinous covering. Pulmonary surface nodular. As her condition grew constantly worse, explorative thoracotomy was performed as the last resort on May 31: All over the lung was covered with small thin-walled cysts, several of which were ruptured. Suture would be impossible as it would have burst the neighbouring cysts. Pneumonectomy was considered contra-indicated, as the lung changes were thought to be bilateral. Autopsy, 3 days later, confirmed this belief. Only a small amount of normal lung tissue was left. Bronchial system normal. Other organs extremely atrophic. Laboratory tests: Wassermann reaction negative. Blood group O. Electrocardiogram: deep S_1 , high R_3 . Blood pressure 105/70. Temperature 37.5—38° C. Pleural effusion amounted to 100—300 ml in the 24-hours, growing less and less hemorrhagic, no growth, no tubercle bacilli upon cultivation. Tuberculin reaction negative.

Summary of the Case: A female, aged 42, had always suffered from dyspnoea and 8 years previously she had had a left-sided spontaneous pneumothorax (with an effusion not analysed at the time). Without previous bodily exertion she got a right-sided valvular pneumothorax and a small, intrapleural hemorrhage. The hemorrhage quickly ceased, but in spite of drainage, re-expansion of the lung and oxygen treatment, a severe dyspnoea persisted. X-ray and thoracoscopy indicated cystic lungs. The diagnosis was proved by an operation, performed as the last resort after the disease had lasted for 19 days and the symptoms grew constantly worse. The cysts were so close together that suture of the numerous ruptures was impossible. Thirty-six hours later the patient died in a state of anoxia. Autopsy showed the cysts in both lungs to be so extensive that the normal lung tissue capable of respiration is reduced to practically nothing. A number of the cysts in the right lung had ruptured. The origin of the hemorrhage could not be demonstrated. There was a universal severe atrophy of the organs.

Case No. 3. (Case rec. 2 18/46). Welder, aged 21. During the last month he had been attending examinations at the Tuberculosis Centre (group survey). X-ray revealed nothing abnormal. Tuberculin reaction was negative. He therefore was subjected to Calmette vaccination on June 14, 1946. On June 23, while he was walking peacefully, he got first pricking and then pressure pain in the right side of the chest. There

had been no preceding cough. The pain was quickly intensified and the patient gradually became short of breath. Five hours after the onset of the disease he was admitted to a fever hospital for pneumonia. Immediately upon admission exsufflation of 1,800 ml of air which was under pressure in the right pleural cavity. This reduced the dyspnoea. On the next morning 1,200 ml were evacuated. In the course of the day his condition again grew worse, and distinct symptoms of anemia supervened. He received two blood transfusions of a total of 900 ml and the same amount of saline solution. In the evening of June 24th the patient was transferred to the Clinic of Thoracic Surgery. Arrived there he was found to be suffering from marked dyspnoea, but no cyanosis (anemia). He exhibited drumstick fingers and curved finger nails. The tension in the right pleural cavity was $+4$, $+12$. The patient was placed in an oxygen tent and given permonid. Thoracocentesis yielding 1,000 ml of a sanguineous fluid (hemoglobin 60 per cent.) and 300 ml of air. Final pressure $\div 6 \div 2$. Prior to the thoracocentesis the ictus cordis had been felt 3 cm lateral to the papillary line, but now it had shifted to 1 cm medial to it. The dyspnoea subsided. On the following days repeated thoracocenteses were performed and gradually the condition was clinically quite satisfactory. For a week the temperature remained between 38° and 39° , whereupon it returned to normal. The tension in the pleural cavity could constantly be kept negative, but in spite of that the lung showed no tendency to full expansion. According to the roentgenograms a mantle of pneumothorax of about 3 cm persisted. After July 1 the effusion was insignificant. The patient was kept in bed during the whole of July, and exsufflations were performed repeatedly. Sufficiency tests showed no signs of the presence of a pulmonary fistula. The tension in the pleural cavity remained fairly constant at about $\div 8$, $\div 4$. In some views of the left lung towards the axilla the roentgenograms showed a couple of fine circular outlines resembling cysts. Right-sided thoracoscopy (on July 29) showed the greater part of the pulmonary surface, particularly in the upper portion, to be the site of numerous cystic prominences, of sizes up to that of a hazel nut. No visible fistula. No adhesions. The pleura looked natural. In order to produce an adhesive pleurisy $\frac{1}{2}$ ml of a 10 per cent. solution of silver nitrate was injected under general anesthesia on August 1. During the following days the tension in the pleural cavity was kept at about $\div 12$, $\div 8$. On the evening of the injection of silver nitrate there was a typical reaction of temperature. The fever rose to 40.1° C. and then followed a lytic fall to normal in

Thoracocenteses in Case No. 3.

No.	Date	Amount	Hemoglobin	Estimated amount of blood loss
1	June 24	1000	60 %	600 ml
2	June 25	550	60 %	330 ml
3	June 26	500	65 %	325 ml
4	June 29	1000	75 %	750 ml
		3050		2005 ml

the course of 9 days. On August 10 X-ray showed a strong broad adhesion towards the axilla. The effusion was insignificant. Without further therapy the lung was now gradually drawn towards the thoracic wall. On August 31 it had expanded completely and the patient was discharged in good health. A monthly follow-up has revealed nothing abnormal, clinically or roentgenologically (apart from the cystic pattern in the lungs). Last follow-up on January 14, 1948. Other examinations: Blood group 0. Pleural effusion sterile. No growth of tubercle bacilli upon cultivation from the pleural effusion and sputum. Mantoux - (100 tuberculin units). Weight 56.7—55.8 kilos. Wassermann reaction negative. Blood pressure 135/75. Sedimentation rate 28—6.

Summary of the Case: Without preceding exertion a male, aged 21, suddenly got a right-sided pneumohemothorax with a severe intrapleural tension which, however, quickly subsided after thoracocentesis. There had been a loss of about 2 litres of blood which was treated with transfusions. Three litres of effusion were evacuated. X-ray as well as thoracoscopy revealed cystic lung. In spite of bed rest, closed pulmonary fistula and a constant negative pressure, the right lung showed no tendency to expand. After having awaited spontaneous expansion for 5 weeks a solution of silver nitrate was injected into the pleural cavity. The result was an adhesive pleurisy with rapid expansion of the lung.

Terminology, Incidence, Etiology, Pathogenesis.

The term spontaneous hemopneumothorax is ordinarily used. Boland (1900) is the only one who uses pneumohemothorax, although this must be said to be the more descriptive of the two. The primary stage of the acute disease is the extravasation of air into the pleural cavity and afterwards the hemorrhage sets in. Of course the disease is not »spontaneous»; there will be a causative disease or an anomaly as well as an accidental cause. Frequently the symptoms arise following exertion or cough (as for instance in the writer's case No. 1). Instead of spontaneous the disease is sometimes called idiopathic (Boland, Hartzell). This should not, however, keep one from always looking for a causative lung disease or anomaly.

In the great majority of cases the syndrome has been observed in men. According to Hopkins only one female has been reported earlier (Hopkins' patient No. 2). Still, Jehn & Sauerbruch have observed the syndrome in a female, affected with pulmonary

metastasis. In both female cases, as in Case 2 reported above, it was a question of quite slight hemorrhage. For the present it is hardly possible to advance any explanation of the numerical male predominance. Spontaneous pneumothorax is about equally common in both sexes. Bodily exertion is hardly a factor of such all-important pathogenetic significance that it could in our days constitute the reason for this great difference. According to general experience, pleural adhesions are equally common in both sexes.

In two of the writer's cases the age is typical: Most cases have been observed in individuals ranging in age from 20 to 30. Between 30 and 35 the affection also occurs quite often. In older persons it is, however, rare. Hopkins' report of 40 cases only included 3 between 35 and 40, and only two of the cases reported in the literature have been older than 40 (Housden & Piggot: 44, Woll: 43). The writer's Case No. 2, a female of 42, is therefore one of the oldest ones. The age distribution among patients exhibiting pneumohemothorax corresponds to what has been observed in spontaneous pneumothorax (Kjærgaard). This conformity also applies to the rare occurrence of the syndrome in persons below 20 years of age. Only Pitt (1 case), Bushby, and Hartzell (2 cases) have observed pneumohemothorax in this age group (18, 17, and 17 years). The syndrome does not appear to have been reported in children.

Pneumohemothorax appears with about equal frequency on the right and left side. In Hartzell's collected statistics of 43 cases 24 were left-sided and 19 right-sided. Of the 29 survivors 19 had the affection on the left side and 10 on the right. In the 14 who died the proportion was reversed: 9 right-sided and 5 left-sided.

The *causative disease* is unknown in the majority of reported cases in which the patients have survived.

Post-mortem examination have, however, almost constantly shown lung changes. Only in Rolleston's and Kiær's cases was it impossible to demonstrate anything abnormal in spite of careful examination and insufflation under water. One case (Davidson's No. 1) exhibited contralateral lung changes, but nothing abnormal on the side of the pneumohemothorax. The remaining 11 non-tuberculous patients dying from pneumohemothorax revealed cicatricial or chronic inflammatory, usually apical lung change, solitary or a few emphysematous, sub-pleural bullae, sometimes ruptured and partly burst adhesions, as a rule to the parietal pleura (Pitt (1st case), Fischer, Housden & Piggot, Davidson

(2nd case), Rossel, Tait & Wakeley, Jones & Gilbert, Louria, Perry, Davidson & Simpson, Lorge).

The *source of the hemorrhage* could only be demonstrated in a few of the cases. In Fischer's case the hemorrhage was considered to have originated in a ruptured large bulla in the pulmonary apex. In the majority of other cases the hemorrhage was considered to come from burst, vascular adhesions. Upon autopsy of the writer's Case 2 the source of the hemorrhage could not be found. This need not, however, be so surprising, as 19 days had elapsed between the onset of the disease and death, and the hemorrhage had been slight.

The possibility that profuse hemorrhage should be able to issue from bullae in spite of their thin walls is ordinarily considered to have been made probable by Mazzei & Pardal (1934) who demonstrated ample vascularization. Persistent hemorrhage from ruptured adhesions is a phenomenon well-known in phthisiology and thoracic surgery. Injury to the intercostal vessels caused by the pneumothorax cannula can be ruled out in the majority of cases considering that the very first puncture has shown a large hemorrhagic effusion.

In order to elucidate the pathogenesis of the hemorrhage the writers has made experiments on a small series of cadavers, stretching adhesions from the lung to the parietal pleura. In 7 out of 10 cases the adhesions at last burst at the site of attachment to the thoracic wall, in 3 cases even tearing a small piece off the parietal pleura. The writer did not succeed in producing unquestionable injury of the blood vessels in the chest-wall in this manner. It is not, however, unreasonable to presume that an injury of *e. g.* an intercostal vein, perhaps in the form of a side hole may result in unfortunate cases. This may result in profuse hemorrhage, and spontaneous hemostasis in the form of a retraction of the vessel will hardly be possible because of the firm site of the vessel in the stiff chest-wall. Autopsies published hitherto do not contradict this hypothesis. The inside of the thoracic wall appears to receive far less attention than the lung in spite of the circumstance that experience always indicates that persistent intrathoracic hemorrhages usually originate in the thoracic wall, perhaps in the mediastinum or diaphragm, and only in comparatively rare cases from the lung (Barrett, Jacobæus, Sauerbruch).

None of the autopsied cases of pneumohemothorax have ex-

disease. When a rupture of a cavity occurs it is nearly always to a pneumothorax space, already established artificially, and the rupture is usually due to necrosis of the lung tissue which covers the cavity. Such necrosis is caused by faulty vascularization and therefore the rupture will hardly ever be attended with hemorrhage. And should there for once be a slight hemorrhage, its symptoms will at any rate be pushed into the background by those of empyema and tension pneumothorax. In a case of cavernous tuberculosis reported by Birch (a), in 1936, the lethal intrapleural hemorrhage appears to have originated in an area of the parietal pleura to which the cavity had been attached.

Another form of hemothorax in pulmonary tuberculosis has been observed rather more often, *i. e.* the form which in exceptional cases follows upon insufflation of a pneumothorax space (Heise & Krause, Hartzell). Injection of a somewhat larger quantity than normal may burst an adhesion and thus cause hemorrhage. Tearing of an adhesion may also occur without injection of air, as in a case, reported by Weiner & Jackson, of contralateral thoracoplasty.

A third form of »pulmonary tuberculosis» which may cause pneumohemothorax is the usually symptomless slight infection which has subsided years ago, leaving only a small apical scar which has entailed the emphysematous bullae mentioned above. This form is, however, not one of clinical tuberculosis, and frequently it is impossible to establish the bacterial etiology of the pathological changes with certainty.

Malignant tumours, so often attended with a sanguineous effusion, very seldom cause the entrance of air into the pleural cavity. Still, Jehn & Sauerbruch have reported one case of pneumohemothorax in a patient exhibiting pulmonary metastasis.

Symptomatology.

Two of the patients with pneumohemothorax had earlier been affected with contralateral spontaneous pneumothorax, Hartzell's 2nd patient and the writer's patient No. 2. During the latter patient's first attack there had been an effusion which was not analysed and which, therefore, may have been hemorrhagic. A search of the literature has not revealed a case of bilateral pneumohemothorax diagnosed with certainty. The same applies

to recurrence of the pneumohemothorax on the same side. A spontaneous pneumothorax perhaps occurred previously in Hopkins' 3rd case. Following pneumohemothorax homolateral spontaneous pneumothorax has been observed by Rist, contralateral by Palmer & Taft and by Rossel. The last-mentioned author's patient died 5 days after his pneumohemothorax from a contralateral spontaneous pneumothorax. In the other cases a period of several years has usually elapsed between the attacks.

Pneumohemothorax is an acute syndrome, sometimes arising in connection with cough, sneezing, laughter, or bodily exertion, and its first symptom is pain. The pain sets in suddenly, it is of a pricking or cutting nature and at the outset often localized to a small part of the thorax (the site where the adhesion has burst). This is at times followed by a brief, subjective improvement, whereupon the pain often returns (the hemorrhage), this time more diffuse, not infrequently extended to the abdomen, more rarely to the precordium or the arm. At the same time the general condition will be influenced by the acute anemia which often acts in the direction of shock. In addition, there will be dyspnoea in a varying degree. The circulation is influenced by the loss of blood as well as the altered intrathoracic pressure.

The pain radiating or localized to the abdomen may often dominate so that in the beginning the pulmonary affection may escape detection. This was what happened in the writer's first case who was hospitalized for a perforated gastric ulcer. The shock-like condition involved by pain and anemia may furthermore misguide one in that direction, and so may nausea and vomiting. Rolleston's patient only escaped laparotomy, because he appeared to be moribund. Fischer's patient was laparotomized. Abdominal symptoms also dominated in the cases reported by Grabfield, Hurxthal, Milhorat, and Hopkins (No. 3).

Precordial pain in connection with the greatly affected circulation aroused suspicion of heart disease in the patient (coronary occlusion) reported by Rist & Worms as well as in the writer's case No. 1. Another misleading factor is radiation of pain to the shoulder, not at all a rare phenomenon, or even to the arm (case No. 2).

The general condition may be dominated by anemia (as in Case No. 1), by dyspnoea (No. 2) or by both (No. 3). The hemorrhage may be quite considerable. In several autopsied cases several litres of blood have been demonstrated in the pleural cavity. In

the survivors too the loss of blood has sometimes been enormous. In one case (Williamson) a total of 10 litres of sanguineous pleural effusion was gradually emptied out, but of course only part of it was blood. In the writer's case No. 3 who was admitted to the Clinic of Thoracic Surgery at an early stage of the acute disease, the hemoglobin percentage in the pleural effusion was determined after each thoracocentesis. By this method the loss of blood has been calculated to have amounted to 2.005 ml of 100 % blood (see the table in the case history). Of this amount 900 ml were replaced by transfusion. The loss of the remaining 1,105 ml caused anemia. If the amount of blood is estimated at 8 per cent. of the body weight (56 kilos), *i. e.* 4,480 g., and if the hemoglobin percentage was 100 prior to the disease, the loss of 1,105 ml ought to cause a fall in the hemoglobin percentage to 75. As stated above it was 72 per cent. Calculated by the method of Aalkjær, this corresponds to a loss of 1,254 ml + the 900 ml which immediately were replaced by transfusion, a total of 2,154 ml, a loss corresponding quite well to the 2,005 ml measured. According to a corresponding calculation patient No. 1 who weighed 74 kilos and who exhibited a fall in the hemoglobin percentage down to 58, must have suffered a loss of about 2.5 litres of blood.

In the majority of cases the hemorrhage has set in soon after the occurrence of the pneumothorax, but according to observations made by Rist & Worms as well as Hartzell it may also be tardive, not appearing until more than 12 hours later. This was what happened in the writer's case No. 3. Of course the symptoms of dyspnoea are of particular violence where the pulmonary fistula acts as a permanent valve. This is particularly harmful and difficult to treat if the lung parenchyma is reduced in volume as in case No. 2.

Fever often attends large hemorrhagic effusions, but it disappears immediately upon thorough removal of the effusion (*cf.* case histories 1 and 3). Drumstick fingers were observed in patient No. 3.

Prognosis.

About one-third of the reported cases of spontaneous pneumothorax have led to death (14 of 43 in Hartzell's series). As a rule it has been the hemorrhage that has proved fatal, partly on account of its effects causing shock and anemia, partly on account of its pressure in the pleural cavity. In one case (Jones & Gilbert)

the hemorrhage was the indirect cause of death which occurred 4 weeks after the onset of the disease, as the enormous masses of fibrin compressed the mediastinal vessels. In other cases the cause of death is anoxia due to the consequences of a tension pneumothorax and — as in case No. 2 — an already existing reduction in the volume of the lung parenchyma.

In the survivors complications are rare. Empyema has been observed by Korol (1st case). A faint, particularly basal, pleural opacity and diminution of the pleural sinuses is a common consequence of pneumohemothorax. It is a sign of adhesive, aseptic pleurisy which must be considered a favourable phenomenon in spite of the fact that it slightly reduces the function of the lung. Fact is that this will reduce the possibility of a recurrence, and if it should happen in spite of all it will probably be less dangerous, because part of the synechia will no doubt persist. It does not, however, constitute any assurance against a recurrence as already mentioned in connection with Rist's patient who exhibited a homolateral spontaneous pneumothorax two years after the attack of pneumohemothorax. Recurrence of the hemorrhage does not appear to have been reported.

Like spontaneous pneumothorax, pneumohemothorax does not often appear to be followed by a later manifestation of pulmonary tuberculosis. Among Hopkins' collected material of 43 cases it was observed in three or four. It is true that most of the patients with pneumohemothorax have only been followed up for a short period, but it is probably warrantable to draw conclusions on the basis of the well-known prognosis of spontaneous pneumothorax found in Kjærgaard's study, considering that the pathological changes in the lungs appear to be identical in the majority of cases.

In all cases reservation must be displayed in judging the prognosis. Recurrences, especially contralateral ones, may occur, and young patients in particular must be warned of the possibility. Moreover, there may be such extensive parenchymatous changes in the lungs — as in the writer's Cases 2 and 3 — that the prospects of life must be considered doubtful in the long run.

Therapy.

During the acute stage the hemorrhage is treated with blood transfusion etc. and the tension in the pleural cavity with ex-

In case of need — severe anemia and no available donor — the blood so evacuated may be used for autotransfusion, if it is emptied out at an early stage. Normally it should not, however, be employed, as it will quickly undergo changes, a fact apparent among other things from the frequent observation of eosinophilia in such blood (Grabfield) and the absorption fever.

As long as the effusion has not been evacuated there is a risk of infection as apparent *e. g.* from a report by Kay & Meade. In the course of a few weeks the precipitation of large amounts of fibrin may necessitate thoracotomy to remove it, a thing often experienced in modern war surgery (Barrett, Thomas & Cleland, Jones, Johnson). Failing an operation, enormous quantities of blood will create a fibrous armour reducing the capacity of respiration and leading to chronic pulmonary changes and disablement.

If the pneumothorax persists in spite of bed rest and exsufflations, one must ascertain the presence of a pulmonary fistula by means of a sufficiency test (measurement of the pressure at varying intervals following exsufflation until a marked negative pressure has been obtained). In case there is a fistula, thoracoscopy is indicated, partly in order to cauterize adhesions, if any, partly to ascertain its site and the condition of the lung on the whole. If no effect is obtained by the cauterization of adhesions, if any, the pulmonary fistula must be closed by producing a chronic, aseptic, adhesive pleurisy, for instance by means of injections of glucose or, probably better still, solution of silver nitrate into the pleural cavity (Brock). This end has also been obtained by surgical procedures (Tyson & Crandall), a method which also has been successfully employed in our Clinic in a couple of cases (Petersen).

If the pulmonary fistula has closed and the pneumothorax persists in spite of all, it is presumably a question of parenchymatous changes in the lung as *e. g.* in the writer's case No. 3. In that case the intrapleural injection of silver nitrate had an excellent effect. The utmost caution must be exercised in performing the injection, particularly in case of cystic lung which is liable to suffer severe damage from the contact with the strong solution of silver nitrate. The lung must be pushed away from the site of injection by means of insufflation and the solution injected into the lowest lateral part of the thorax with the patient lying down.

Summary.

The writer submits three atypical cases of the so-called spontaneous pneumohemothorax. In the first case the disease was first interpreted as perforated gastric ulcer, then as heart disease. The symptomatology of the other two was typical, but X-ray, thoracoscopy and thoracotomy revealed bilateral cystic lung, a causative disease which does not appear to have been reported earlier.

On the basis of experiments on cadavers and a review of some 50 cases of this syndrome reported in the literature, the writer advances the hypothesis that in most cases the hemorrhage is due to injury to blood vessels in the thoracic wall. Such injury is considered to be caused by rupture of the parietal attachment of adhesions following primary tension pneumothorax.

Addendum.

After the conclusion of this article the writer has treated a case of chronic pneumohemothorax. A man, 39 years of age, was admitted to a psychiatric ward for morphia abuse. His complaints were dyspnoea and pains in the left side of the chest. Four years previous the patient had a spontaneous pneumohemothorax. He was treated half a year by bed-rest. The blood was not evacuated. Tuberculosis was not demonstrated. Now the left side of the chest was retracted and x-ray gave evidence of a limited pneumothorax. The pressure was 0. Exsufflation was not possible, the lung being un-expandable. A leftsided thoracotomy was performed. The lung was compressed by a firm thick visceral pleura with blood-pigment in the basal parts. After decortication the lung promptly reexpanded. The patient has now been well for six months. The dyspnoea has subsided, the ability to hard work has returned, and there are no more pains in the chest. The patient does not use morphia or other analgetics.

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On the Treatment of Heart Block with Adrenergic Substances.¹

By

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During the last 4 years I have had occasion to employ adrenaline or sympathicomimetic amines in the treatment of cardiac syncope, manifest or approaching, brought about by ventricular standstill or bradycardia from heart block. As the knowledge of this form of treatment hardly is widespread and as the histories of some of these patients to me seem rather fascinating, an account will be given of the experiences gained.

Previous Investigations.

Since about 1920, an increasing number of papers have been published on the treatment of the Stokes-Adams syndrome with adrenaline and related substances.

In 1913 Cullis & Tribe showed that when cats, in whom auriculoventricular block had been produced by division of the bundle of His, were given adrenaline intravenously, both the auricular and the ventricular rates were accelerated. This phenomenon has been demonstrated by several other authors in experiments on dogs (Egmond, Routier and Hardoy & Houssay). Thus Routier succeeded even in obtaining a transitory restoration of the normal mechanism — presumably the block in his experiments produced by clamping of the bundle of His, was

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merely incomplete. Besides the accelerating effect of adrenaline on the action of the heart, it is to be mentioned that in these animal experiments also the occurrence of extrasystoles was observed, indicating that a state of hyperexcitability of the heart was produced. In his experiments, Kahn (1909) observed even sometimes complete auriculoventricular block, presumably a reflex vagus effect. The doses of adrenaline employed in these animal experiments were $\frac{1}{10}$ — $\frac{1}{20}$ mg for dogs of medium size.

Without knowledge of these experiments, Danielopolu & Danulescu (1915) as the first clinicians gave adrenaline to a woman with incomplete heart block. The result of this was an acceleration of the rate of the auricles and ventricles, independent of each other, and then abolition of the block. In another patient with complete block, this treatment gave only the usual increase in the rate of the auricles and ventricles but no change in the block. Since then, numerous clinical papers have been published on this subject. Here it will suffice to give a brief and somewhat schematic survey of the literature accessible to me. For details, the reader is referred in particular to the papers by Phear & Parkinson (1922), Feil (1923) and Gilchrist (1934 and 1945).

1. Subcutaneous (or intramuscular) injection of adrenaline in doses of 0.3—0.5 mg, most often several times daily at suitable intervals, is able in certain cases of heart block, especially in acute block, to reestablish the normal heart rate.

2. Even though restoration of the normal mechanism is not obtained, most often there will still be an acceleration of the auricular and ventricular rates.

3. Generally, according to Gilchrist, it may be said that the lower the initial value, the greater is the increase in the ventricular frequency, and usually the same effect is obtained, whether the dose employed is 0.25, 0.50 or 1 mg adrenaline.

4. Through the effect of adrenaline on the heart it is possible to abolish the Stokes-Adams syndrome or prevent its reappearance. Also a pronounced tendency to frequent attacks of the Stokes-Adams syndrome may be brought to a stop even though the block is not abolished, and even though the greatly reduced ventricular frequency is not increased merely by the slow rate becoming stabilized.

5. Although the Stokes-Adams syndrome often shows a tendency to self-limitation, the various papers on adrenaline therapy in such cases leave a strong impression of the mentioned results

being »propter» rather than »post». This is suggested especially by those cases in which the treatment was instituted only after observation for days or even for months.

6. The effect of adrenaline may be explained in three ways:

a. By direct action on the sympathetic endings in the auricles and ventricles — according to the most recent view, by direct effect on the muscle cells.

b. By sensitization of the specific conductive system.

c. By stimulation and stabilization of new secondary centers of stimulation in the ventricular musculature.

Since 1925 also ephedrine and near-related substances with prolonged adrenergic effect — more recently, amphetamine (benzedrine, »mecodrin») — have been mentioned in connection with the treatment of Stokes-Adams syndrome (20, 14).

As to untoward effects and contraindications, these points will be taken up later on, in the discussion of my own cases.

Writer's Cases.

Case 1. (Record No. 942/44.) Med. Dep. B, Copenhagen County Hosp. Woman, aged 66, widow of minister. Admitted 26/11/43—1/2/44.

In the last half a year the patient has become somewhat short of breath on climbing stairs. Four months before admission, 2 attacks of dizziness of the ship's deck type. The patient was confined to bed for a few days, whereafter she was feeling relatively well. About 10 days before admission, again an attack of dizziness, now followed by vomiting and syncope. The summoned physician counted a pulse rate of 4 per min. After subcutaneous injection of 0.5 mg adrenaline, the attack subsided rapidly. Shortly after, the pulse rate was 16, a little later 30 and finally 60, at which level it kept staying. The first few days the temperature was about 38°, but there was no anginal discomfort. When, after 10 days' confinement to bed, she tried to get up, there was at once an approach to syncope, with a pulse rate of about 30. These phenomena subsided rapidly after she had returned to bed. Then she was admitted to the hospital.

Physical Examination: Appearance corresponding to her age, not distressed or exhausted, without any sign of cardiac insufficiency. Mental habitus normal. Pupils, throat and tongue normal. Teeth poorly, partly replaced with prothesis. Moderate degree of alveolar pyorrhea. Palpation of the neck: No abnormality. Auscultation of the heart: Relative borders slightly extended; sounds clear; action regular, 52 per min. No peripheral arteriosclerosis. Lungs: No abnormality. Abdomen: No abnormality. Extremities thin; no edema. Feet and hands warm. Reflexes normal.

Special Examinations: Height: 156 cm. Weight: 47.4 kg. Urine: No albumin, pus or sugar. Diuresis: 300—600 ml; sp. gr.: 1024—1006.

Temperature normal. Hemoglobin (Sicca): 92 %. Sedimentation rate 5, 6, 5 mm. White blood count: 7800—8200. Differential count normal. Antistreptolytic titer: 64 (normal). Blood pressure: 155/70 mm Hg. Basal metabolism (Benedict & Harris): 96 %. Roentgenography of the heart: Form normal; width: $4.2 + 8.9 = 13.1$ cm; length: 14.3 cm; width of the thorax: 23.7 cm. Aorta measured in oblique diameter: 3.9 cm. Special otologic exam.: Bilateral cochlear neuro-labyrinthopathy.

Electrocardiography, Course and Treatment.

On admission, there was sinus rhythm, but in the following 2 days the pulse rate varied, round 40 per min. In the evening of 28/11 an attack of dizziness, with a pulse rate of 30. Electrocardiography showed complete auriculoventricular block. (Fig. 1.)

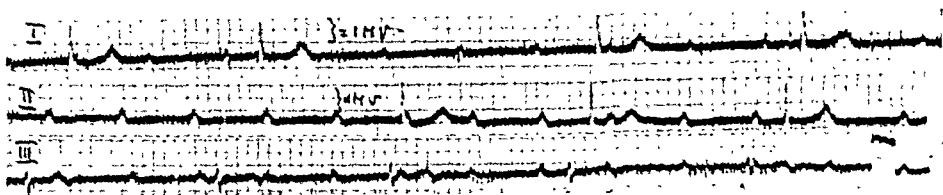


Fig. 1. Case 1. Complete block. Ventricular rate varying from about 19 to 36. Auricular rate varying from 85 to 91.

Ephedrine (5 cg) had an excellent effect on her condition. The pulse rate rose to 64. The treatment was continued with ephedrine, 2.5 cg \times 2 daily. In addition, the patient was given phenemal, 1.5 cg \times 3 and 10 cg in the evening. Electrocardiography, 10/12: Normal findings, except for slight *left* axis deviation. The following days, again dizziness, especially at noon after dinner. From 14/12 the ephedrine treatment was continued with 2.5 cg \times 3 daily. From 16/12 she was also given oxedrine, 20 drops \times 3. Pulse regular, 36. Electrocardiography: Complete block, auricular rate of about 86; ventricular rate about 35. On 18/12 dizziness in connection with bed-making, a little later, involuntary urination. The patient became cool and pale, with imperceptible pulse. The nurse, as directed beforehand, gave her at once 0.5 mg adrenaline subcutaneously. 10 min. later the pulse rate was 16, and a little later it was 32. Now ephedrine and oxedrine were discontinued and it was decided to try amphetamine, which at first was given in a dosage of 5 mg daily. The following 2 days, the pulse rate was about 20—22. On 20/12, in the morning the pulse rate was 16, irregular, with pauses up to 8 sec. The patient was then given an intravenous injection of sol. aminophyllini fort., 2 ml (= 48 cg aminophylline) together with 18 ml 20 % glucose solution. Immediately after this injection the pulse became regular, 32 per min. The dose of amphetamine was then increased to 5 mg \times 3, given at 8, 12 and 15 o'clock. After 2 days of this treatment the pulse was regular, about 60 per min., and kept at this level during the rest of her stay in the hospital. But there still remained a tendency to bradycardia and dizziness. These phenomena were provoked by slight exertion or emotional agitation, and they became particularly frequent when the patient began to get up, after 5 weeks'

confinement to bed. From 13/1/44 the treatment was continued with 4 amphetamine tablets daily, under which the patient again improved. At her discharge from the hospital she was directed to continue with 5 amphetamine tablets daily: 2 on getting up in the morning, 2 at noon, and 1 at 15 o'clock. Together with this she was given a larger dose of sedatives: phenemal, 3 cg \times 3; diemal, 25 (50) cg in the evening; if necessary, supplemented with 1 evipane tablet. In addition, on account of constipation, from which she had not been suffering previously the patient was directed to take 2 cascara tablets daily. The blood pressure, which was measured regularly, did not exceed 160/70 mm Hg; the lowest value measured was 100/70 mm; usually the systolic pressure was about 140 to 150 mm.

Readmission, 20/4—10/6/44.

The patient was getting along nicely after her discharge, with only 3—4 attacks of dizziness. The reason for her readmission is that since her discharge she has been troubled with increasing rheumatoid changes in the finger joints, wrists, elbows, shoulders and knees.

Physical Examination: Findings similar to those described before. Electrocardiogram normal. Blood pressure: 200/100 mm. Roentgenography of the heart: Same as before, but measurement of the aorta in oblique diameter was now 4.5 cm.

The main interest was now attached to the joints, with severe arthrotic changes in both hands. All fingers semiflexed in the basal and interphalangeal joints, a normal extension of the fingers impossible. The skin of the fingers was atrophic, tightly bound to the skeletal parts («glove fingers»). The skin was warm as normally. The mobility in the right carpal wrist was greatly reduced, in the left only moderately. Only slight changes in the other joints. Oscillometry of the upper and lower extremities normal.

Reexamination, in October 1945 (after nearly 2 years' treatment): The patient is still feeling well. Occasionally, when she is worried or

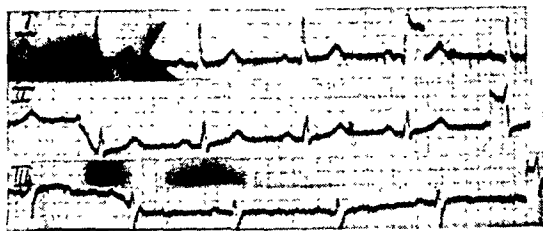


Fig. 2. Case 1. Normal rhythm. Frequency ca. 70 per min. Slight axis deviation to the left.

tired, she has an attack of dizziness. The same happened one day when she had not taken the amphetamine tablets. She has lost about 2 kg. in weight. Auscultation of the heart as before. Blood pressure: 175/95 mm. Electrocardiogram normal, except for slight axis deviation to the left (see Fig. 2).

Later it has been learnt that about 6 months after the reexamination, the patient was admitted to a surgical clinic for uncertain abdominalia. Shortly after, she died at home with increasing ascites of uncertain cardiac origin. Most often the rhythm remained normal.

Case 2. (Record No. 1996/44.) Med. Dep. B. Copenhagen County Hosp. Man, aged 63, married, slaughter. Admitted 22/10—8/12/44.

During the last half year, increasing attacks of angina pectoris. After an episode, 3 months before admission, when he became greatly agitated because his shop was under German fire, his cardiac attacks have increased markedly in severity. On 20/10 he had an attack of violent pain that commenced in the evening and lasted all night. Not until the following morning did he call a physician who gave him an injection of morphine, which was repeated later in the day. After this, he was fairly comfortable until 22/10, when he had a new attack of pains, accompanied by dyspnea. He was very restless, got out of bed—and tumbled to the floor. Another summoned physician gave him an injection of tetrapon, but found that further observation at home would be justifiable. During the following 5 hours before his admission to the hospital, his condition was very poor, with precordial pain, dyspnea, restlessness and tendency to unconsciousness. At 15 o'clock he again was given an injection of tetrapon, and at 16.10 he entered the hospital.

Physical Examination on Admission: Very restless, dyspneic, pale-cyanotic. Extremities chilly. Pulse variable, at times imperceptible for 15—30 sec., then irregular, slow, changing to brief periods in which it was quite palpable and regular. Whenever the pulse became imperceptible, the patient became unconscious, the respiration stopped, and this was accompanied by increased cyanosis and frothing at the mouth. The limbs became rigid, with slight tonic convulsions, but no clonus was seen. He was sweating profusely.

Auscultation of the heart: The borders appear not to be definitely enlarged. Sounds distant, but clear. Action markedly irregular.

Otherwise no abnormalities revealed. Looks corresponding to his age. Nutrition fair; habitus pyenic.

Electrocardiography, Course and Treatment.

Immediately after his admission, the patient was given an injection of 1.5 cg morphine hydrochloride and an injection of 2 ml nikethamide. Before further treatment was given, an electrocardiogram was taken (Fig. 3).

As was expected the electrocardiogram showed a complete auriculo-ventricular block of varying frequency. On account of the continually recurrent attacks it was rather difficult technically to obtain nice tracings. After an attack of unconsciousness, we succeeded in taking a brief period with regular pulse frequency (Fig. 4).

This was a series of ventricular contractions, presumably produced from a new ventricular automatic center of stimulation.



Fig. 3. Case 2. *Complete auriculoventricular dissociation.* Ventricular frequency varying from about 13—30. Auricular frequency 109. R_1 in initial complex Nos. 2 and 3 is 3—4 mm high, followed by a U-formed S wave. Width of initial complex 0.16 sec. Initial complex II and III: 0.10—0.12 sec. T_1 negative. T_{II} and T_{III} high, in several places concealing the P wave.

At 18.15 the patient was given 0.6 mg adrenaline subcutaneously. 5—10 min. later the pulse was regular, 104, but kept in this way only for a few minutes, whereafter an attack of block could be observed in the searcher of the electrocardiograph. At 18.30 the patient was given

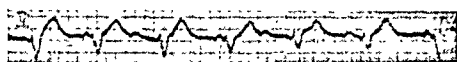


Fig. 4. Case 2. Lead II. *Spontaneously changing pacemaker.* Initial complexes looking entirely different from those in Fig. 3. R waves quite small, 2—3 mm, and S waves very deep. Ventricular frequency about 86. Auricular frequency about 120.

0.6 mg adrenaline intravenously, after which the pulse rate at once was 132, regular. Then an electrocardiogram was taken in Lead II (Fig. 5).

In Fig. 5 we see a number of ventricular systoles elicited from different ventricular centers of stimulation, activated by the intravenous

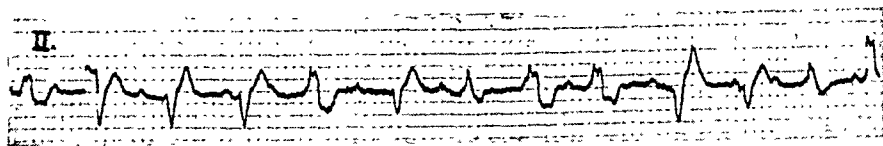


Fig. 5. Case 2. Lead II. *After intravenous injection of 0.6 mg adrenaline.* At least 5 different types of ventricular complexes. P waves cannot be followed with certainty. Ventricular frequency about 76.

administration of adrenaline. At this point of time, without knowing the electrocardiogram, the clinical judgement was that a normal rhythm had been reestablished. During an attempt to take electrocardiograms in all 3 extremital leads, the block and syncope reappeared. In this state a series of auricular contractions were taken while there was complete standstill of the ventricles for at least 18 sec. (Fig. 6).

The ventricular standstill was followed by complete block with uniform ventricular complexes from a new stimulation center (Fig. 7).

Now the patient was given an intravenous injection of sol. aminophyllini fort. (= 48 cg) + 18 ml 20 % glucose solution. Within 5—10

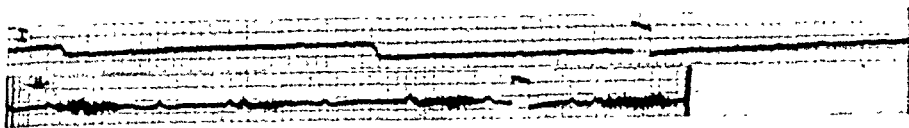


Fig. 6. Case 2. Leads I and II. ECG. taken during ventricular standstill for 18 sec. Auricular frequency in Lead I about 128; in Lead II about 65. In Lead II some rhythmic fibrillary contractions.

min. the pulse became regular. Of his own accord, the patient stated that he was feeling better: the precordial pressure he had been suffering for a couple of days was now gone. His condition became more stable. The pulse was 52—44, regular. A new electrocardiogram showed block with complexes essentially as on admission, though now T_I was posi-

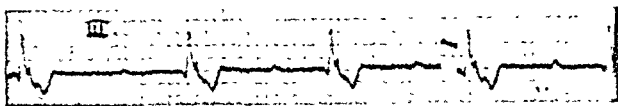


Fig. 7. Case 2. Lead III. New ventricular complexes. P waves partially concealed in the initial complexes. Ventricular frequency about 38.

tive. From about 19 o'clock the heart action was practically regular, but without sinus rhythm at any point of time. The block varied continuously, and «new» ventricular contractions were observed (see Fig. 8).

During the first 4 days in the hospital the pulse was counted every half hour. Now the pulse rate varied from 48 to 28. At 19, 21 and 22

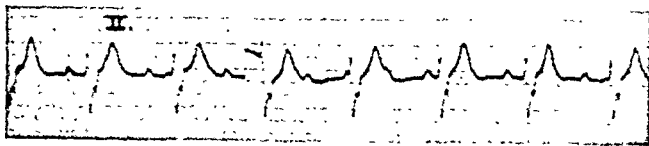


Fig. 8. Case 2. Lead II. New ventricular complexes. Ventricular frequency ca. 53. Auricular frequency ca. 120.

o'clock, the patient was given 5 cg ephedrine. At 22 the blood pressure was 80/55 mm mercury. At 23, again syncope. Adrenaline, 0.6 mg subcutaneously had no effect. Likewise aminophylline + glucose (same dose as above). Then 0.6 mg adrenaline was given intravenously. Now the pulse became regular, 92. Blood pressure 110/70. The patient awoke again. Half an hour later the pulse rate was 52, and during the night it fell to 40. The following morning, 23/10 the electrocardiogram (Fig. 9) still showed complete block, but now also signs of infarction of the anterior wall. After midnight the patient had been resting relatively well on a total dose of 2.5 ml hypnofen. At the ward-round he answered clearly to questions. Looks somewhat pale, with slight cyanosis of the lips, but no dyspnea. Moderate hiccough. Extremities warm. Auscultation of the heart: Nothing new. Lungs: scattered ronchi on the posterior surface. Pulse rate during the following 18 hours 48—40. At 13 o'clock

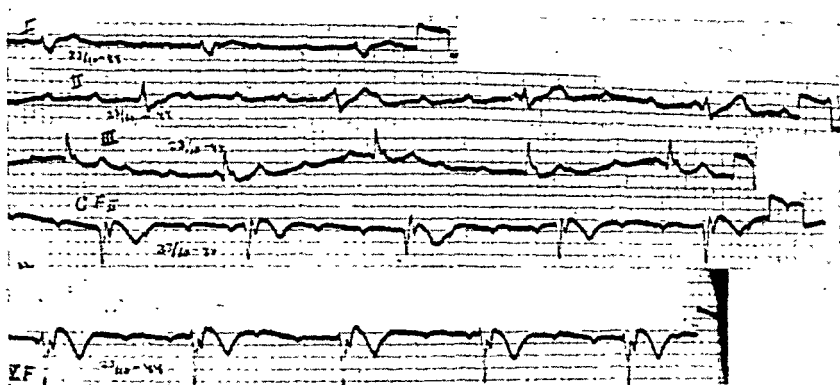


Fig. 9. Case 2. ECG. taken on the day after admission. *Signs of anterior wall infarction.* Initial complexes essentially as on admission (Fig. 3). S—T_I interval now slightly elevated, however, and S—T_{II}—S—T_{III} slightly depressed. Q wave in CF₂ large. T waves in extremal leads positive, in chest lead negative. Also P waves negative in chest lead. Ventricular rate 30—35. Auricular rate ca. 120.

10 mg amphetamine sulphate was given, and this was repeated 1 hour later. Besides, the patient was given, as daily prescription, 5+5+10 centigram phenemal; in the evening an injection of 2 ml hypnofen + 0.5 ml 2 % dilaudid.

On 24/10, at 3 a. m. the patient was somewhat restless, pulse 104, during the next 6 hours 100—90, then falling gradually to 60—70. The pulse was at first somewhat soft, varying a little, but sinus

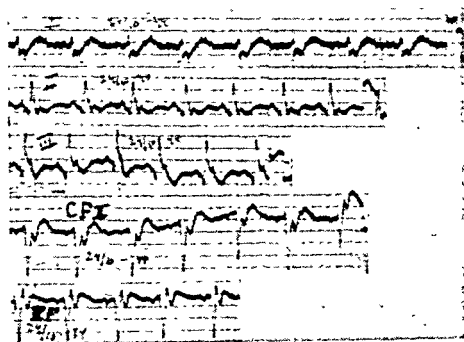


Fig. 10. Case 2. *Full rhythm.* Initial complexes and T waves undergone a marked change. Rate 102.

rhythm was reestablished, and it persisted. Electrocardiogram on 24/10 (morning) showed normal rhythm (fig. 10).

Auscultation revealed pericardial friction sounds.

Since admission, no anginal pain. Clinical course typical of coronary occlusion. Temperature on admission subnormal, 36.8° in evening, rising in 24 hours to 38.3°. During the following 4—5 days it was about 38°, then subfebrile for 12 days, and then normal. Parallel with the rise

in temperature there was slight leucocytosis (15,040—10,000/cmm) and increased sedimentation rate (14—65—30—15 mm). The diuresis was very scanty on admission, later normal, sp. gr. 1.024. Blood pressure, as mentioned, low on admission, later 110/65—105/65. Urine: No albumin, sugar or urobilin. Blood urea on admission 105 mg%, next day 63 mg%, 4 days later 53 mg%, on discharge 27 mg%. Plasma bicarbonate and serum chlorine: Normal values. Ophthalmoscopy: No abnormality. Hemoglobin (Sicca): 100—93 %. Wassermann negative. Kahn negative.

At first electrocardiography was performed daily, later twice a week, and finally once a week. Continuous sinus rhythm. Clinically, some-

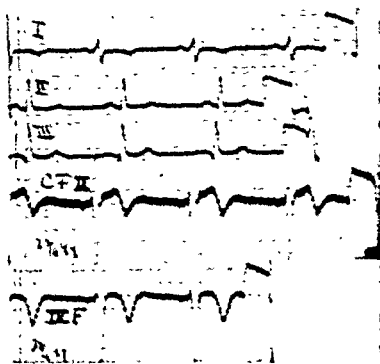


Fig. 11. Case 2. ECG. at discharge. *Signs of consequences from anterior wall infarction.* T_I negative, T_{III} positive. Deep central curve in CF_{II} and $IV F$. No sign of intraventricular conductive impairment. Rate 67.

times extrasystoles. ECG. at discharge: features typical of passed anterior wall infarction (Fig. 11).

Treatment, apart from what has been mentioned already: 6 weeks' bed-rest, moderate reduction in fluid and caloric intake.

About 2 weeks after his discharge from the hospital the patient returned in the ambulance — dead. He had been feeling well at home, but lately he had had considerable worries concerning his business. On Christmas day he suddenly became very ill. The summoned physician gave him tetrapon and nikethamide, but he died on his way to the hospital. No information could be obtained about the pulse rate at the time of the attack.

Autopsy (Dr. Soeborg Ohlsen):

Heart: Weight 470 g. *Pericardium:* The lower part of the anterior wall and the almost entire posterior wall firmly attached to the pericardium through fibrous and fibrinous adhesions. *Myocardium:* Corresponding to the entire septum and lower part of the anterior wall, the musculature has been replaced with fibrous tissue. *Coronary arteries:* Pronounced atherosclerosis, with complete occlusion of the left descending branch at some distance from its origin. Ram. circumflex. greatly narrowed; its descending branches completely occluded. *Endo-*

cardium: In the left ventricle, towards the apex, a few small parietal thrombi. *Lungs*: Large, hyperemic and edematous; some mucopurulent fluid in the air passages.

Case 3. (Record No. 289/43.) Med. Dep. B, Copenhagen County Hosp. Man, aged 62, pensioned fireman. Admitted 9/2—25/2/43.

Since 1931 the patient had been troubled with increased blood pressure, dizziness and tiredness, aggravated periodically. In 1939, hospitalized twice. Electrocardiography showed slow sinus arrhythmia; rate varying between 33 and 52. Roentgenography of the heart: Width 18 cm. Thorax: 30 cm. Treated with atropine pills 2×3 , with good effect. Blood pressure 165/80—175/80 mm. Wassermann negative; Kahn negative.

Present Admission: Complaints of attacks of dizziness, not gyratory, lasting only a few seconds. Sensation of standstill of the heart. Dyspnea from the slightest exertion.

Physical Examination: Looks corresponding to his age. Nutrition fair. No sign of cardiac insufficiency. Small xanthelasmata on the upper eyelids. Pupils, throat and neck normal. Auscultation of the heart: Borders greatly enlarged, 2 cm to the right; ictus almost in the anterior axillary line; systolic blowing at the apex, pulse regular, 40. Lungs: Scanty crepitation over the lower posterior surfaces. Electrocardiography shows slow sinus arrhythmia. Heart rate varying between 38 and 46.

Course and Treatment.

During his stay in the hospital, several approaches to fainting. The patient was given 1 ephedrine tablet of 5 cg daily, later 1 tablet $\times 2$. Then the attacks ceased. The pulse rate kept at about 40. Discharge from hospital with prescription of $\frac{1}{2}$ tablet $\times 2$.

Reexamination (3 months later): Feeling relatively well, taking now the tablets only periodically when he gets dizzy and feels like fainting. He has noticed himself that the symptoms appear when the pulse rate falls below 28. On intake of $\frac{1}{2}$ ephedrine tablet $\times 2$ daily the pulse rate rises within a few days to 50—60.

On physical examination the findings recorded were the same as previously. Now, however, *electrocardiography showed fibrillo-flutter*. Ventricular rate variable, 28—83. At no point of time could an electrocardiogram be taken under the attacks of block.

Reexamination: June 1946: Feeling better. Dizziness of rare appearance, sometimes at intervals of months. Now capable of even considerable exertion — in his vacation, for instance, he climbed a hill, 148 m in height!

Physical examination showed the same features as before. Blood pressure: 220/110 mm.

Case 4. (Record No. 170/46). Med. Dep., Silkeborg City and County Hosp. Woman, aged 48, single, head-nurse. Admitted 3/1—25/2/46.

At the age of 18, rheumatic fever, with confinement to bed for 3 weeks; joint affection not particularly pronounced. In September to October 1942 hospitalized under the diagnosis: Mitral stenosis. The symptoms were bronchitis, dyspnea and a single nocturnal attack of dyspnea. Electrocardiography: Delayed conduction, 0.32 seconds; low T_I ; slight axis deviation to the left. Roentgenography of the heart: slight enlargement of the heart, triangular in form. Blood pressure 150/90 mm. Hemoglobin (Sahli) 65—83 %. Sedimentation rate 10 mm. During the following 3 years, feeling relatively well. After a forced journey at New Year 1946 she was troubled with some dizziness and had a rather severe attack of epigastric pains, radiating out in the interscapular region and lasting several hours, on which account the patient was admitted to this department.

Physical Examination: Looks a little old for her age. Nutrition over medium. No cyanosis, dyspnea, anemia or icterus. Pupils, throat, neck normal. Auscultation of the heart: Borders slightly enlarged. Over the entire precordium a typical crescendo sound; second sound snapping. At the left sternal margin, gallop rhythm. P_{II} accentuated. Action irregular as in extrasystole. Lungs: No abnormality. Abdomen and extremities, normal findings. *Special Examinations:* Temperature normal. Diuresis and specific gravity, normal. Urine: No albumin, pus or sugar. Height: 157 cm. Weight: 63.3 kg. Blood pressure 115/75 mm. Roentgenography of the heart: Width (distance of 1.5 m) 13.4 cm; thorax: 25 cm; slight protrusion of the shadow corresponding to the left auricle.

Electrocardiography, Course and Treatment.

As previously, ECG. showed a prolonged P—R interval of slightly varying length (0.30—0.36 sec.) with Wenckebach periods. The ventricular rate varied between 26 and 60. The pulse rate was highly variable during the first part of her stay in the hospital, with a tendency to fall to 40 or lower. During such periods the patient would be feeling poorly. This tendency to bradycardia yielded to treatment with atropine (0.5 mg \times 3—4), and then the pulse rate was about 70. As the atropine treatment was uncomfortable to the patient because of dryness of the mucous membranes, it was replaced with «mecodrin» (amphetamine), 5 mg daily, in the morning. The patient has since (at this writing, for about one year) been taking 1—1½ sometimes 2 mecodrin tablets daily, in the forenoon, and she has been feeling well. Only seldom has she had a sensation of skipping pulse or bradycardia. When she does not take the tablets, she has bradycardia and general malaise, sometimes a sensation of oppression.

Case 5. (Record No. 941/46.) Med. Dep. Silkeborg City and County Hosp. Woman, aged 55, wife of sawmill superintendent. Admitted 5/8—8/10/46.

2—3 years ago onset of illness with dyspnea and tendency to fainting. The fainting spells irregularly, often at very long intervals, sometimes appearing several times in the day, and occurring at night too. For

1½ years the patient has been treated with thyroidin and phenemal. In addition, she has been given ephedrine, 5 cg \times 3, which she thinks has had a particularly good effect on her condition. Further, the dizziness has subsided even more in the last 1½ months, while she also was given amphetamine, 5 mg \times 3 daily, before 14 o'clock.

Physical Examination: Nutrition over medium. Slight cyanosis of the lips. Slight polypnea. Pupils, throat and tongue normal. Venous pulsation on the neck. Auscultation of the heart: Borders enlarged 1 cm to either side. Slight systolic blowing over the precordium. Action regular, 48. Lungs: A few hypostatic râles. Abdomen large and obese, without signs of passive congestion. Slight edema of the legs.

Special Examinations: Temperature normal. Diuresis and sp. gr. normal. Urine: No albumin, pus or sugar. Height: 159 cm. Weight: 69.4—66 kg. Hemoglobin (Sicca): 105 %. Sedimentation rate: 11 mm. Wassermann negative; Kahn negative. Antistreptolysin titer: 0. Blood pressure: 190/90—130/80 mm. Roentgenography: Width of the heart (at a distance of 1.5 m) 16 cm. Thorax: 28.5 cm. The dilatation involves chiefly the left ventricle. Basal metabolism (Benedict & Harris): 101 %.

Electrocardiography, Course and Treatment.

Rest in bed for 37 days. No attack of dizziness or fainting here. Pulse rate on admission 52—40; then falling slowly in 2 weeks to 32—28. After treatment with ephedrine, 2.5 cg \times 3, and mecodrin, 5 mg \times 5 for about 3 weeks, the pulse rate slowly rose to 40—52. The blood pressure kept normal from 2 weeks after admission. After discontinuance of ephedrine the pulse rate fell again to 32—36. Suitable sedatives and hypnotics were given at the same time. Throughout the stay in the hospital the electrocardiograms showed complete auriculoventricular block of the same appearance. The T waves were at first a little low, later of the coronary type, positive in Lead I, negative in Lead III; T_{II} slightly negative.

Adrenaline test with 0.5 mg adrenaline subcutaneously:

	Ventricular rate	Auricular rate	Blood pressure
Before inj.	about 32	about 39	125/90
After »	» 38	» 71	135/60

Electrocardiography and measuring of the blood pressure every 2—5 min. The comparative values here recorded were measured 25 min. after the injection.

Case 6. (Record No. 598/46.) Med. Dep., Silkeborg City and County Hosp. Man, aged 71, married cotter. Admitted 9/5—22/6/46.

2½ years ago the patient commenced being troubled with functional dyspnea and tendency to dizziness, also a few fainting spells.

On admission to this hospital 2 years ago electrocardiography showed complete auriculoventricular block, together with hypertension, 185—175/90—85 mm. Since then, his physician has occasionally given him ephedrine, with some favorable effect on the dizziness. Now he is ad-

Electrocardiography, Course and Treatment.

The patient was admitted at 13 o'clock and immediately given 0.5 mg adrenaline subcutaneously; 10 min. later, 0.5 mg intravenously. The pulse rate rose from 30 to 70, feeling regular, and then fell abruptly again to 30. There was no time for electrocardiography then. The blood pressure rose from 150 to 200 mm, systolic. Then, euphyllin-glucose (in the aforementioned dosage) was given intravenously, besides 500 ml glucose-saline subcutaneously, hot-water bottle, and adrenaline, 0.5 mg intramuscularly. The unconsciousness lasted till 20 o'clock, when the patient awoke and was clear. Blood pressure, 145/90 mm. Electrocardiography showed complete auriculoventricular block with ventricular and auricular rates of 24 and 92, respectively. Then an intravenous injection of ephedrine (5 cg) was given, whereafter the ventricular and auricular rates rose to 36 and 109, respectively; the blood pressure to 180 mm.

Physical Examination on the Day after Admission: Slight orthopnea, pronounced cyanosis. Auscultation of the heart: Ictus 3 cm outside the midclavicular line. Sawing systolic murmur over the entire precordium. No thrill or bulging of the precordium. Lungs: Numerous ronchi over all lung fields. Abdomen: Liver extending one hand's breadth below the costal margin. Extremities: Rather considerable edema.

Special Examinations: Temperature essentially normal. Hemoglobin (Sicca): 87 %. Urine: No albumin or sugar. Wassermann negative; Kahn negative. Blood urea: 141 mg%. Plasma bicarbonate and chlorine: Normal values.

Course: Repeated attacks of dyspnea of asthmatic character. Adrenaline relieved one of these attacks. Venesection was performed with evacuation of 420 ml. Increasing haziness, with Cheyne-Stokes respiration. Exitus on the 3rd day in the hospital.

Autopsy (Dr. Søbørg Ohlsen):

Hypertrophy and dilatation of the heart. Fibrosis of the myocardium, diffuse and localized. Mitral stenosis. Chronic endocarditis of the mitral valve. Atherosclerosis of the coronary arteries and aorta. Nephrosclerosis. Chronic passive congestion of organs. Purulent tracheo-bronchitis. Congestion and edema of the lungs. Acute gastro-enteritis, severe degree.

Discussion.

In the present material the therapeutic effect of adrenergic substances on heart block has been about as mentioned in the introduction. The prominent effect of adrenaline in acute cardiac syncope from extreme bradycardia or ventricular standstill is plainly evident from Cases 1 and 2, the effect proved particularly

striking in Case 2 where a number of syncopees unceasingly recurring for hours, were checked by administration of adrenaline.¹

An approach to syncope in the form of dizziness due to bradycardia is seen to have been dealt with effectively in Cases 3, 4, 5 and 6. In Case 7 treatment with adrenergic remedies was not tried till the heart lesion had persisted for a long time, and, in addition, it was complicated by uncompensated mitral stenosis, acute gastro-enteritis and renal insufficiency. Presumably, however, the adrenaline therapy has contributed to carry the patient through the state of shock in which he was brought to the hospital. Still — in contrast to the first two cases this case cannot be said to furnish a striking proof of the effectivity of the adrenaline therapy.

No essentially new contribution to the explanation of the action of the adrenergic remedies has been given by the cases mentioned. The present material confirms the clinical experience mentioned in the introduction: that adrenergic substances, especially in acute cases of complete block, are able to reestablish full rhythm. All the present cases confirm the capacity of adrenergic remedies for acceleration of the auricular and ventricular rates — though in unequal degrees. Only in Case 6 could no effect of this kind be demonstrated with certainty. But, then, in this case only one trial was made with adrenaline (injected subcutaneously). This trial was not repeated because of the appearance of intraventricular conductive inhibition — a point that will be taken up later on. Presumably the mechanism in the action of the adrenergic remedies is to be looked upon partly as sensitization of the conductive bundle (Case 1 and Case 4), partly as a direct effect on the auriculoventricular musculature (remaining cases).

Case 2 gives some valuable information about the working mode of the heart under an attack of Stokes-Adams syndrome. A few similar cases have been described in the same way. Schwartz

¹ After the conclusion of this paper, I have had occasion to try the employment of adrenaline in one additional case of cardiac syncope due to heart block.

This was the case of a man, 51 years old, in whom the heart block arose in connection with acute coronary occlusion (verified electrocardiographically). (Record No. 137/47. Med. Dep., Silkeborg City and County Hosp.)

Injection of adrenaline gave a typical acceleration of the ventricular rate from 20 to about 50 and, on relapse, from about 3 to 30—40. Still, the patient died.

Since this paper was sent to the editor, in this department we have had 6 additional cases of heart block. In 3 of these, with ventricular standstill, adrenaline had a definite effect; in 2 cases (terminating fatally) it had no effect; and in 1 case there was a dubious effect from sympathicomimetic amines on the dizziness produced by extreme bradycardia in complete auriculoventricular dissociation.

(1936) has even published 15 cases with auriculoventricular dissociation in acute coronary occlusion. Among 45 cases of Stokes-Adams syndrome observed for 4 years, 15 were due to the consequences of acute coronary occlusion. Our own Case 2 illustrates the most common mechanism in cardiac syncope: the ventricular standstill. The ventricular complexes of differing appearance found in the various electrocardiograms signify the activity of secondary centers of impulses. It is worth emphasizing that these complexes were observed even before the commencement of the adrenaline therapy, that is: they arose spontaneously. As early as 1920, without employment of any electrocardiographic technique, Lumbacher called attention to the occurrence of spontaneously changing ventricular contractions in heart block. Herapath (1926), employing an electrocardiographic technique, has pointed out this »shifting of the ventricular pacemaker» in connection with attacks of Stokes-Adams syndrome.

As to the numerous different ventricular »extrasystoles» that appeared after intravenous injection of adrenaline in Case 2, they will be discussed later on.

There can be no doubt that the observed effect of the treatment in Case 2 was due to stimulation and stabilization of new ventricular centers of impulses that prevented a new ventricular standstill. In this way the cerebral anemia was abolished, and consciousness returned. The state was characterized not only by the heart block but also by an initial shock associated with a low blood pressure. This pressure gradually became normal and then kept at a normal level. It is rather likely that the blood pressure had been increased before the coronary occlusion set in. Thus all the patients in Schwartz' material presenting this syndrome had hypertension before the coronary occlusion appeared.

Perhaps the intravenous injection of euphyllin-glucose solution played some rôle in the results obtained in our first mentioned two cases. An undeniable favorable effect on the clinical condition of the patients was observed in Case 1, and also after the first injection in Case 2. This may be interpreted by the well known dilating effect of euphyllin on the coronary vessels.

It is rather surprising that full rhythm could be re-established in Case 2, as here the entire septum was ischemic and, later, underwent fibrosis. This shows that, on account of collateral edema or spasm of the supplying vessels, the auriculo-ventricular node was affected but temporarily. In this connection it will be appropriate

to point out that clinically it was a matter of an anterior wall infarction. A priori, one would have expected a posterior wall infarction, as in more than 90 % of the cases examined the auriculoventricular node receives its blood supply from the posterior coronary system. Autopsy, however, revealed extensive occlusion in both the anterior and posterior coronary system. Particular mention is to be made of the fact that the first electrocardiogram taken shows evidence of intraventricular conductive disturbances. The same applied also to several of the patients in Schwartz' material.

Naturally adrenaline has been employed in the acute situations, while the sympathicomimetic amines with weaker, but prolonged effect are given through a longer or shorter period in order to stabilize the results obtained (Cases 1 and 2). Furthermore, these remedies have been employed as the only form of adrenergic treatment continuously in Cases 4 and 5, merely periodically in Cases 3 and 6.

A priori we had no idea that our amphetamine therapy would be so protracted as in Case 1. But, as the condition of this patient under the development of the complete block each time was very poorly, it seemed necessary to continue the treatment, even though it is the prevailing view that once the block becomes permanent, it gives no symptoms in the form of syncope or approach to such. If the adrenergic therapy had given any particular untoward effects it would have been obvious to try to let the block stabilize spontaneously on a physiological ventricular automatism.

We then meet with the question: What risk is associated with the administration of adrenaline and kindred remedies to patients with coronary affections — as most commonly encountered in these patients?

In principle the administration of adrenaline produces indeed a rise in the blood pressure and thus an increase in the work of the heart, accentuating presumably — through reflex action — the vagal constrictor effect on the coronary vessels, although the primary effect of adrenaline itself here is vasodilation. According to V. Larsen the other commonly employed and near-related amines have a constrictor effect on the coronary arteries. Presumably this quality may be left out of consideration as these remedies in the dosage here employed are not likely to reach such a concentration in the blood as employed by Larsen in his experiments on rabbits. On our patients anginal pain has not been

observed in connection with the adrenergic therapy. Naturally, if anginal distress had been observed in a patient, the therapy would have been abandoned.

As mentioned already, adrenaline has been found able in animal experiments to produce heart block. Clinically, in one case, an incomplete block has been seen temporarily to change into complete block (Korner & Christie), but on careful dosage such cases appear to be very rare. For the sake of accuracy, it is to be mentioned that in the present material, patient No. 6 showed slight signs of intraventricular disturbances on the day after the adrenaline test. It is doubtful, however, whether this phenomenon was due precisely to the administration of adrenaline.

On discussing the untoward effects, it is of particular interest to return to Case 2, in which intravenous injection of 0.6 mg adrenaline was followed by the appearance of numerous »extrasystoles». The resulting condition has been observed also by previous authors. Thus Lutembacher (1920) advised against intravenous injection of adrenaline because of the subsequent occurrence of extrasystoles, which in his cases was followed by syncope and aggravation of the block. (In my own Case 2 a single relapse of syncope also was observed after the first intravenous injection of adrenaline.) In Denmark the capacity of adrenaline for production of extrasystoles has been observed by Siggaaard Andersen in patients with diphtheric myocarditis. Nathanson has designated this condition very characteristically as prefibrillation of the ventricles. Thus, this therapy may give rise to a ventricular hyperexcitability, analogous with the form of paroxysmal ventricular tachycardia which, after Gallavardin & Froment, is designated as prefibrillary ventricular tachycardia — a condition involving the risk of turning into ventricular fibrillation. It has to be admitted that I did not realize the actual danger to our patient involved in the adrenaline dosage mentioned till I had studied the electrocardiogram in question. Judging from the literature, however, several other clinicians have been equally aggressive in their therapy. According to our present experiences, we will have to be content with subcutaneous or intramuscular injection of adrenaline (0.3—0.5 ml of a 1 ‰ adrenaline hydrochloride solution), possibly repeated at suitable intervals. If the condition is very threatening — as in our case — in future we will follow the principle suggested by Gilchrist (9) and inject merely 0.05 mg intravenously (0.5 ml 1 ‰ adrenaline solution + 4.5 ml

saline, 0.5 ml of which is slowly injected intravenously). If no effect is obtained with this dose, undoubtedly it will be safe to repeat it or even increase it somewhat. In desperate cases with circulatory standstill Gilchrist advises intracardial injection of 0.25 mg adrenaline.

Also the rare cases of Stokes-Adams syndrome due to auricular standstill — the highest degree of sino-auricular block — are to be looked upon as a strong indication for adrenaline therapy. Whereas this treatment naturally will be contraindicated in the relatively rare instances of Stokes-Adams syndrome due to ventricular fibrillation. So, when faced by a fully developed instance of Stokes-Adams syndrome, in which it may be justifiable to postpone the treatment, an electrocardiogram ought to be taken before any injection of adrenaline is given. If the attack is due to ventricular fibrillation it is advisable to try intravenous injection of quinidine sulphate in the dosage given by Trier & Siggaard Andersen in the treatment of paroxysmal tachycardia: 50 cg intravenously¹. As a prophylactic measure against a tendency to such attacks, quinidine sulphate may be given in a dose of 20 cg \times 3—4. Possibly it may be of advantage in such cases to try 15 mg mecholyl (acetyl-beta-methyl-choline) injected subcutaneously or intramuscularly (perhaps intravenously?). Nathanson (19) has been able experimentally to show that adrenergic remedies produce a certain degree of ventricular hyperexcitability in elderly persons in whom he had produced ventricular standstill by pressure on the carotid sinus. When these patients beforehand were treated with quinidine or mecholyl, the extrasystoles otherwise produced by the adrenergic remedies failed to appear.

Considering finally the less dramatic by-effects of adrenergic therapy, Case 1 in our material is to be pointed out because this patient was treated through a period of about 2 years with large doses of amphetamine. The effect on the blood pressure was relatively slight. There was no mental by-effect in the form of undesirable emotional stimulation. It is to be mentioned, however,

¹ Since this paper was sent to the editor, in a few cases I have tried quinidine sulphate intravenously for ventricular fibrillation with fatal result. If the remedy is to be employed in such cases, presumably it will have to be given in smaller doses, and the injection has to proceed exceedingly slowly. In such cases it might be advisable to try procain intravenously — in view of the experiences reported with this substance in ventricular fibrillation occurring on the operating table (cf. E. R. Ruzicka & M. J. Nicholson: *Cardiac Arrest under Anesthesia*, J. A. M. A. 135, 622, 1947).

that a not inconsiderable amount of sedatives and hypnotics were given at the same time. The possibility cannot be excluded altogether that amphetamine may in part have been responsible for the development of the polyarthrosis that appeared in patient No. 1 (adrenergic vascular effect?). Of additional by-effects of amphetamine in this patient, attention may be called to a constipating effect due to stimulation of the intestinal sympathetic plexus.

Summary.

A survey is given of the accessible literature on the effect of adrenergic remedies in heart block.

Abstract is given of the case records of 7 patients with different forms of heart block in whom adrenergic therapy of differing kind and intensity was tried.

The principle of adrenergic therapy in heart block is stimulation of heterotopic ventricular pacemakers and sensitization of the auriculoventricular conductive bundle.

Among the treated cases, two are to be pointed out in particular. In connection with the development of complete auriculoventricular dissociation, one of these patients had bradycardia or ventricular standstill with cardiac syncope that yielded promptly to 0.5 mg adrenaline given subcutaneously. Normal rhythm was produced and maintained through about 2 years by means of 25 mg amphetamine daily. The patient died presumably from some non-cardiac disease. In the other patient a complete auriculoventricular block developed in connection with acute coronary occlusion. The clinical condition of this patient was characterized by pronounced shock and numerous syncopes due to ventricular standstill. The attacks were checked by injections of adrenaline. In this way a certain basal rhythm was established that was stabilized with sympathicomimetic amines. About 32 hours later full rhythm. This case of heart block was followed electrocardiographically throughout the resulting Stokes-Adams syndrome.

Intravenous injection of 0.6 mg adrenaline was seen to be followed by numerous ventricular extrasystoles of varying origin, signifying the appearance of an excessive ventricular hyperirritability (ventricular prefibrillation).

The writer advises against an excessively high dosage of adrenaline, as this may involve a fatal risk. It is recommended to follow

that a not inconsiderable amount of sedatives and hypnotics were given at the same time. The possibility cannot be excluded altogether that amphetamine may in part have been responsible for the development of the polyarthrosis that appeared in patient No. 1 (adrenergic vascular effect?). Of additional by-effects of amphetamine in this patient, attention may be called to a constipating effect due to stimulation of the intestinal sympathetic plexus.

Summary.

A survey is given of the accessible literature on the effect of adrenergic remedies in heart block.

Abstract is given of the case records of 7 patients with different forms of heart block in whom adrenergic therapy of differing kind and intensity was tried.

The principle of adrenergic therapy in heart block is stimulation of heterotopic ventricular pacemakers and sensitization of the auriculoventricular conductive bundle.

Among the treated cases, two are to be pointed out in particular. In connection with the development of complete auriculoventricular dissociation, one of these patients had bradycardia or ventricular standstill with cardiac syncope that yielded promptly to 0.5 mg adrenaline given subcutaneously. Normal rhythm was produced and maintained through about 2 years by means of 25 mg amphetamine daily. The patient died presumably from some non-cardiac disease. In the other patient a complete auriculoventricular block developed in connection with acute coronary occlusion. The clinical condition of this patient was characterized by pronounced shock and numerous syncopes due to ventricular standstill. The attacks were checked by injections of adrenaline. In this way a certain basal rhythm was established that was stabilized with sympathicomimetic amines. About 32 hours later full rhythm. This case of heart block was followed electrocardiographically throughout the resulting Stokes-Adams syndrome.

Intravenous injection of 0.6 mg adrenaline was seen to be followed by numerous ventricular extrasystoles of varying origin, signifying the appearance of an excessive ventricular hyperirritability (ventricular prefibrillation).

The writer advises against an excessively high dosage of adrenaline, as this may involve a fatal risk. It is recommended to follow

the dosage given by Gilchrist: 0.5 mg subcutaneously — if required, repeated suitably. In more severe cases as the present: 0.05 mg intravenously, and in desperate cases with circulatory standstill: 0.25 mg intracardially.

In the more infrequent cases of Stokes-Adams syndrome due to ventricular fibrillation treatment with adrenergic remedies is contraindicated.

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From the First Medical Clinic, Sahlgren's Hospital,
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(Head: Professor M. Odin, M. D.)

Spontaneous Hypoliquorrhea.

Report of a Case.

By

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(Submitted for publication June 1, 1948.)

The increased cerebrospinal pressure often noted in connection with lumbar puncture has been the subject of much study. Considerably less attention has been paid to the conditions associated with a marked decrease in pressure. It has, however, been known for a long time that a low cerebrospinal pressure not infrequently follows head injuries, operations on the brain, spinal anesthesia, and lumbar puncture, and that low pressure may also arise in connection with a subdural hematoma. In recent years, Schaltenbrand, in particular, has made a study of these conditions, and his collaborator, Wolff, has published a monograph on the subject (1). The fact that certain characteristic symptoms must be attributed to low cerebrospinal pressure in itself was, as a matter of fact, demonstrated in a convincing and effective manner by Ingvar (2) as far back as 1923.

Schaltenbrand (3), basing his statements on three cases which he himself had encountered, described a special syndrome, for which he suggested the name »spontaneous aliquorrhea». These three cases were described and discussed in more detail by Wolff.

Later, Puech, Perrin and Koechlin (4) published a report on some cases of meningitis and a case of encephalitis with low cerebrospinal pressure, but it is very uncertain whether these cases belong to the same group as those mentioned by Schalten-

brand. Hesser (5, 6) has described two cases which undoubtedly must be counted as belonging to this class.

The subjective symptoms in these cases consisted of headache, tinnitus, and dizziness. One of the most characteristic features was that the symptoms became aggravated when the patient was in the erect position but were less severe or absent in the recumbent position. Lumbar puncture revealed a low or a negative cerebrospinal pressure, and in consequence of this it was in some cases difficult to obtain enough fluid for study. In all cases hitherto examined the cerebrospinal fluid was blood-tinged and yellowish. There was an increased concentration of protein but little or no increase in the number of white cells. At the neurologic examination Wolff found slight differences in the reflexes between the right and left sides in one patient, but in other respects no pathologic signs were detected. The patients were afebrile, and in most cases the sedimentation rate was normal. In one of Wolff's patients, however, the sedimentation rate was 25 mm in 1 hour. The blood pattern appeared to be normal. In all cases the patients recovered. There are no autopsy reports on record.

We have had the opportunity to study a case which, in our opinion, belongs to this syndrome.

Case report. E. A., a baker's messenger aged 38.

In 1928 and 1932 he was operated on for a gastric ulcer. After the second operation he had no more trouble from his stomach.

While doing his compulsory military training, around Feb. 12, 1945, he began to feel weary, and at times he experienced a prickling sensation in his back and chest. On Feb. 23, he developed a headache, and thumping pains all over his head. He vomited and felt sick but felt no dizziness. He noticed that his symptoms disappeared when

he was lying down, but that the symptoms returned when he was sitting or standing up.

He was admitted to the hospital on March 26, 1945, and while lying in bed he had no symptoms whatsoever. When he stood up he had a troublesome headache but no other symptoms. The routine examination revealed nothing of interest. The sedimentation rate was 4 mm in 1 hour. White blood count, 6,000.

The neurologic examination revealed that his neck was slightly stiff. Kernig's sign was positive on both sides at 80 degrees. There were no pareses, no sensibility disturbances, and no cerebellar or extrapyramidal phenomena. The reflexes were normal.

As meningitis was suspected, a lumbar puncture was carried out on Feb. 28. The patient was lying on his side. There was no doubt but that the needle perforated the dura, but no fluid ran out. When

the patient was brought into a sitting posture the fluid in the pressure column rose but when he lay down again the fluid was sucked back in its entirety, and a sucking noise indicated that air was also passing in. There was thus a negative pressure in the vertebral canal. With the patient in the sitting posture the pressure rose when Queckenstedt's test was performed.

Only a couple of cubic centimeters of fluid were obtained. The fluid drawn was slightly blood-tinged. It contained 15,300 red blood cells to each 3.2 cubic millimeters. The majority of these were pineapple-formed. The white blood count was 24 to each 3.2 cubic millimeters, 16 of these being mononuclear and 8 polynuclear cells. The amount of fluid obtained was not sufficient to allow a quantitative protein analysis to be made.

Lumbar puncture was carried out repeatedly. The results are shown in table 1.

Table 1.

Date	Pressure in recumbent position	Fluid obtained ml	Red blood cells per 3.2 mm ³	White blood cells per 3.2 mm ³		Protein %
				poly-nuclear	mono-nuclear	
28/2	neg.	3	15,300	8	16	
8/3	neg.	12	960	0	10	0.30
20/3	neg.	13	230	24	230	1.21
6/4	1 cm	20	26	2	30	0.30
16/4	7 cm	12	10	8	30	0.30

In connection with the lumbar punctures carried out on March 8 and March 20, no fluid was obtained when the patient was in the recumbent position but when he was in the sitting posture the fluid in the column rose. When he lay down again all the fluid together with air were sucked in through the needle. In order to make quite sure that this finding was not due to chance he was made to sit up and lie down again three times at each examination. The result was consistently the same.

When the above-mentioned punctures were carried out we had not made any arrangements for measuring the negative pressure. At the examination made on Apr. 6 the initial pressure was 1 cm. After we had withdrawn 20 ml of fluid, with the patient in the sitting posture, the pressure when the patient was in the recumbent position was measured as — 11 cm of water with the aid of a fluid manometer.

During the entire course of his illness the patient was afebrile. He suffered from a slight headache but had no other symptoms. His neck was slightly stiff, however, until the beginning of April. His symptoms did not increase in severity in connection with the lumbar puncture.

He was discharged free of symptoms on Apr. 19, 1945.

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From the Medical Department of the Central Hospital at Falun, Sweden.

Haverhill Fever.

(In Connection with a Case Observed in Sweden.)

By

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(Submitted for publication June 10, 1948.)

As early as in 1914 Schottmüller made the communication that bites of rats and mice could infect human beings with a bacillus discovered by him, *streptobacillus moniliformis*, and give rise to an acute septic morbid condition with exanthema and articular symptoms. This illness is regularly mentioned in the ordinary American textbooks and handbooks, under the same head »Rat-bite fever», as the well known »Sodoku» caused by infection with spirochete morsus muris, with which it not only has the mode of infection in common but also exhibits definite similarities in the morbid picture.

A less known but more important fact is that milk infected with *streptobacillus moniliformis* may give rise to sudden epidemics, and that isolated cases appear from quite unknown infection sources. These modes of infection are not met with in cases of spirochetetal infection.

The first proved epidemic of this nature was that in Haverhill, Mass., U. S. A. described in 1926 by Place and Sutton, which comprised 71 cases, all occurring within a very small district of the town. All the 39 households infected had bought milk from the same dairy. The milk was not pasteurized. The initial source of infection could not be traced. Place and Sutton described the illness as erythema arthriticum epidemicum or »Haverhill fever». The latter name has received acceptance, probably in the first place owing to the varying clinical picture, which is difficult to

cover with one descriptive name. The incubation period was taken to be 1—3 days.

As mice, and especially rats, proved to be the natural carriers of streptobacillus moniliformis, and the bacteria are met with abundantly in their urine, it was considered probable that the milk had been infected by rat urine.

Place and Sutton point out that an epidemic at Chester, Pa. in 1925, described by Armstrong and Wood and comprising 400 cases, appears to tally with the Haverhill epidemic in respect of distribution, clinical course and epidemiological relation to milk. It was not possible to establish what micro-organism caused the disease in the Chester epidemic, but in that case the possibility of streptobacillus moniliformis had been overlooked.

Earlier and subsequent publications on the subject all deal with isolated cases or inconsiderable group infections. Of the older works, that of Levaditi, Nicolou and Poincloux is worthy of being saved from oblivion. It was published as early as in 1925 and was then unique, inasmuch as there was no rat-bite in the case described. The latter exhibited three well-delimited phases, with shivering-fits, fever, severe headache, articular symptoms and exanthema, while the fever receded. By means of streptobacillus moniliformis from the patient injected intra-articularly or intravenously, Levaditi and Schoen could with great certainty elicit acute or chronic generalized polyarthritis in mice, in which animals they could also prove spontaneous polyarthritis of this etiology. Levaditi and Schoen produced an infection in rabbits with symptoms which strongly recalled the syndrome in man (even exanthema was present).

The micro-organism. This was proved with great regularity in blood and articular punctates from infected persons and is a common finding in the nasopharynx, and in the urine of rats and mice. In the case of Swedish rats and mice, S. Gard stated that, in his studies of infantile paralysis, he had found streptobacillus moniliformis in the intestine of the albinotic and the wild rat rat in about 2/3 of the animals examined.

Streptobacillus moniliformis will probably be the most pleomorphic micro-organism known on the whole and thus presents considerable theoretical interest. The pleomorphism has naturally led to somewhat varying, sometimes contradictory, descriptions of that bacterium. The designations met with in the literature, streptobacillus moniliformis, haverhillia multiformis, strepto-

thrix muris ratti, *streptothrix putorii*, etc., will thus probably at most indicate different strains. The bacterium is classed among the family *Mycobacteriaceae*. It is non-motile, gram-negative and stains unevenly. Its most characteristic mode of growth (which has given rise to the name) is in long chains of more or less clearly marked rods, on which are seen knot-shaped swellings »like knots on a rope«. At other times it has a more fusiform appearance, resembles spermias or grows in the form of shorter and longer rods lying in heaps. Finally, it may appear in the completely different L-forms tallying with the pleuro-pneumonia bovis group (Klieneberger).

The microbe grows poorly or not at all (Brown and Nunemaker) on ordinary agar and broth. In the case of hemocultures in broth with abundant amounts of blood it does not grow until after 48 hours, according to Levaditi, and the colonies then are in or on the blood coagulum, and therefore the broth does not become cloudy. Thus the growth readily escapes detection. Further, owing to its pleomorphic appearance, the *streptobacillus* may readily be taken to be an impurity.

Schottmüller and Levaditi gave the fundamental contributions to the biology of *streptobacillus moniliformis* and called attention to the rôle of rodents as virus reservoirs and transmitters of the infection. More detailed bacteriological knowledge was arrived at by Parker and Hudson. These researchers worked up the Haverhill epidemic bacterioserologically, prepared suitable substrates and introduced the agglutination reaction with rabbit serum. Of later authors, special mention may be made of Brown and Nunemaker as the source of detailed bacterioserological information on *streptobacillus moniliformis*. According to these authors, the bacteria grow like streptobacilli (of very varied appearance) in characteristic colonies under optimal conditions. In old cultures and, for the rest, under poor growth conditions, the so-called L-form (Klieneberger), suggestive of pleuro-pneumonia bovis,¹ appears. Brown and Nunemaker consider that, in the case of infected persons and animals, *streptobacillus moniliformis* appear, especially in the last-mentioned form. These colonies exhibit an appearance diverging from the *streptobacillus* form, and the very small individual elements are in the shape of irregular grains or form a fine network.

¹ Pleuro-pneumonia bovis gives rise to pneumonia in cattle. It is a transition form to virus, owing to its ultrafiltrability, but grows in cell-free media.

formis known in Scandinavia. On the two occasions when I spoke of my observation in lectures, the above work had not yet been published.

In the Medical Department at Falun we have recently observed a case which, although streptobacillus moniliformis was not proved, may with the greatest degree of probability be classified as a case of Haverhill fever, in view of both the clinical picture and the results of the penicillin therapy, as well as the strongly positive result of the agglutination reaction carried out by Dr Brown, Washington. A discussion with my friend Prof. Jan Waldenström contributed effectively to the investigation of the case.

E. U. B. (case records 1428/47) a 19-year-old girl living at home in the parish of Ore in north Dalecarlia. As early as at the age of 8 years inflammation of the kidneys, with albumin and blood in the urine. Confined to bed for a couple of months and subsequently permanently free of albumin. In 1939 otitis media sin. No albuminuria in connection therewith. In May or June 1944 languid and tired. Consulted a doctor, who found albuminuria again. Nursed in the Medical Department at Falu Hospital 27/12 1944—18/1 1945 for slight chronic glomerulonephritis with good kidney function.

The illness proper: Returned home a week before the onset from a week's visit to a young people's camp. During the immediately preceding period she had not been outside her home district. No similar case there or in the patient's family or in the neighbourhood was known. Is unaware of having been bitten by a rat.

She was admitted to the Medical Department at Falu Hospital on 7th August 1947 after having been ill for 5 weeks. Fell ill with shivering-fits, temperature of 40° C, severe headache and backache. After 24—48 hours eruption on the extensor sides of the arms and legs and a falling temperature. These attacks had been repeated, at intervals of a week, four times altogether, when she was admitted to the hospital.

When admitted (7/8) she was afebrile and subjectively free of trouble. Sedimentation reaction 114 mm, white blood corpuscles 10,800 (3 % eosinophil). On the extensor sides of the lower arms some inconsiderable pale red efflorescences.

On 10/8 pain in the ankle-joints and in the right thigh. Slight swelling of both talocrural joints and great tenderness on pressure over the femoral musculature, where it is thought a firmer tender area can be felt.

11/8 Shivering-fit, 40° C, severe headache.

12/8 Extensive exanthema on the extensor sides of the arms and legs, consisting of efflorescences the size of confetti and larger, some with a small hemorrhagic centre, some somewhat infiltrated. On the same day the morning temperature was 37.5. White blood corpuscles 20,000.

13/8. Fresh rise in temperature and pronounced stiffness of the neck. In the afternoon of this and the following two days the patient is somewhat severely affected. Lumbar puncture revealed raised pressure (260 mm), greatly increased albumin content, and 12,900 cells per mm³, 10,400 of which lymphocytes. The cerebro-spinal fluid sugar 0.023 % (blood sugar 0.146 %). No bacteria in direct preparations or in cultures with ascites broth. After 1/2 hour a spider's web coagulum is formed.

Already on the following day the changes in the cerebro-spinal fluid were less pronounced, but the rise in temperature still persisted.

15/8 penicillin was begun, 240,000 units per 24 hours. After another shivering-fit with a rise of temperature the temperature becomes permanently normal within a week (22/8) (her seventh peak of Haverhill fever with the same periodicity as earlier but without exanthema?). During the whole of her remaining stay of more than 1 month in hospital the patient was troubled by articular symptoms: pain and stiffness in the shoulder joints, joints of the hands and ankle-joints. On several occasions appreciable swelling in the ankle-joints was established. The symptoms from the last-mentioned joints still persisted at the end of October. The other symptoms disappeared very rapidly with penicillin treatment.

No persisting exacerbation of the nephritis appears.

At a follow-up examination on 29/1 1948 even the articular symptoms had disappeared.

Laboratory Examinations.

Blood cultures (agar plates, broth) negative 12/8 and 17/8.

Lumbar punctures

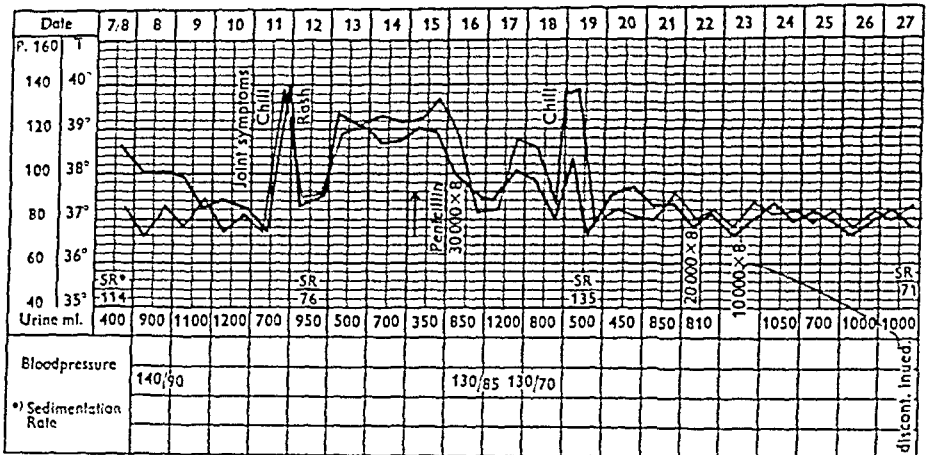
Date Aug.	Pressure	Pandy	Nonne	Spider's web coagulum	White/mm ³	Leuko-cytes	Lymphocytes	Sugar %	
								Cerebro-spinal fluid	Blood
13	260	+++	+++	+	12,900	2,500	10,400	0.023	0.146
14	210	+++	+++	—	4,100	1,000	3,100	0.059	0.189
19	170	+++	+++	—	2,600	1,700	960	0.064	0.169

Cultures on ascites broth with negative results on every occasion.

Fluid from the puncture on 14/8 was sent to the State Bacteriological Laboratory for inoculation on guinea-pigs and cultures on tubercle bacteria, with negative results.

The serum was examined for the occurrence of agglutinins against salmonella, brucella and tularensis bacteria and against Weil spirochetes with negative results.

The polyarthritides symptoms led to the determination of antistreptolysins, firstly on 15/8 and secondly during the convalescence, 19/9,



with results of 220 and 440 units per ml respectively, and streptococcus agglutination, with the result agglutination in the dilution 1/40 (Lancefield A).

On 6/9 I sent serum for agglutination against streptobacillus moniliformis to Dr. Frank Horsfall at the Rockefeller Institute in New York and wrote, *inter alia*, the following: »We have not been able to find an explanation for this unusual clinical picture, but feel that the case in several respects bears a resemblance to the textbook descriptions of streptococcus moniliformis infection. This disease being unknown in this country we would very much like to know whether our suspicion could be confirmed or disproved.» In his turn Dr. Horsfall sent our serum to Thomas McPherson Brown, Washington, the previously cited authority on the subject. Brown found the agglutination »extremely high», namely the titer 1/20,480 (1/40,960 negative).

Thus I consider I have diagnosed a sporadic case of infection with streptobacillus moniliformis, Haverhill fever, without any known mode of infection — the first in Sweden. The fever exhibited a pronounced recurrent typical course. In other respects also the symptom picture was classical, with typical exanthema and articular symptoms in the correct relation to the temperature curve. Acute lymphocytic meningitis appeared as a complication, whether caused by the basic illness or by a secondary (virus?) infection is left an open question.

With penicillin treatment rapid recovery, except for long persisting slight articular symptoms.

I have considered it especially urgent to direct attention to this infection illness, the rarity of which is probably due, at least partly, to the fact that it is extremely easily overlooked. Firstly, with the present blood culture technique at Swedish hospitals there will be great possibilities the attempts to get the bacteria

to grow will fail, secondly, lack of acquaintance with it and its pleomorphism will probably readily result in any growth being dismissed as an impurity. Further, it is probably easy to label the case incorrectly, *e. g.* as febris rheumatica with erythema exsudativum multiforme.

A more extensive use of blood cultures on special substrates (see in the first place Brown and Nunemaker) and of the agglutination reaction would probably soon result in the detection of fresh cases. At the present time the Swedish State Bacteriological Laboratory is preparing the serobacteriological diagnostics.

Finally, streptobacillus moniliformis infection should be of the greatest interest for experimental rheumatology.

Summary.

The author gives a short description of the infection with streptobacillus moniliformis and deals with its three principal epidemiological features.

- 1) Rat-bite fever.
- 2) Epidemic infection by food, generally named Haverhill fever.
- 3) Sporadic cases of Haverhill fever with unknown mode of infection.

He claims to have established the diagnosis of a sporadic case, the first one described in Sweden.

The discovery by S. Gard of streptobacillus moniliformis as an inhabitant of the intestinal tract of Swedish rats indicates that the possibility exists of the infection of man in this country.

Penicillin treatment in this case seems to have been successful.

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From the Medical Clinic of the University of Lund, Sweden.

On the Artificial Kidney VI.

Some Views on the Indications for Treatment of Uremia
and for Active Removal of Oedema by Means of our
Artificial Kidney, Based on Studies of Uremic
Material not Treated with this Method.

By

NILS ALWALL and BIRGER HERNER.

(Submitted for publication March 5, 1948.)

The chance of removing a possibly fatal uremia by dialytic treatment has produced augmented demands for an adequate estimation of the prognosis of uremia. It is true, general information on the course and prognosis of uremia can be obtained from handbooks and other sources, but naturally they do not consider the new eventualities, created by dialytic therapy. In the following we put forward some views on the indications for this treatment of uremia and for removal of oedema with the aid of our »artificial kidney», based on the study of the earlier material of the clinic which was not treated with dialysis.

Primary material: Table 1 gives data of acute and chronic cases of nephritis from this clinic in the last 17 and 12 years respectively.

Conforming with general experience there are few deaths, 3, at blood non-protein nitrogen (N.P.N.) levels below 100 mg %. In these 3 cases of chronic nephritis death can be explained by causes other than uremia.

For the final study only the cases with N.P.N. above 100 mg % are of interest to us. Of the acute nephritides with such a rise of N.P.N. level, about 50 % have died, of the chronic nephritides about 40 %.

As is shown by table 2 several cases have been excluded for different reasons; with their elimination the data seems more suitable to illustrate our problem.

Table 1.

The table gives the material of the clinic for the duration of time mentioned, from which the cases discussed in this publication have been chosen.

Nephritis	N.P.N. ≤ 100 mg %		N.P.N. > 100 mg %	
	Survivals	Deaths	Survivals	Deaths
Acute (subacute) . . .	280	0	21	19
Chronic	281	3	29	46

Table 2.

The table shows the shifting of the uremic cases with a blood-N.P.N. level above 100 mg % in order to get a material, where uremia can be assumed to have been the essential cause of death, or has been so severe and protracted that the case can be regarded as suitable to illustrate our problem.

A. *Acute nephritis, survivals:*

Total number	21
Excluded: Empyema pleurae	2
Temporary rise of N.P.N.	3
Short time of observation	3
Remaining cases	13

B. *Acute—subacute nephritis, deaths:*

Total number	19
Excluded: Uncertain infection, hepatitis etc.	1
Sepsis	2
Mediastinitis, empyema pleurae	1
Hypertonia, cirrhosis hepatis	1
Vitium organicum cordis, diabetes mellitus	1
Remaining cases	13

C. *Chronic nephritis, deaths:*

Total number	46
Excluded: Endocarditis lenta	1
Pneumonia	3
Insufficiencia cordis	3
Short time of observation or incomplete examination	13
Other causes	2
Remaining cases	24

The cases left for study thus consist of 13 patients with acute nephritis, who survived uremia with N.P.N. above 100 mg % and 13 who died of acute—subacute nephritis. As in this connection the deaths are of greater interest, we have enlarged this

group with 3 cases from older records of the clinic and — by the courtesy of Professor Malte Ljungdahl, M. D. — 4 cases from the Malmö clinic. Thus, this group contains no less than 20 cases.

Of the chronic nephritides only 24 cases, who died of uremia with N.P.N. levels above 100 mg % are discussed.

The amount of urine, its specific gravity, blood-N.P.N., blood-pressure, pulse, and temperature are reported in the following. Other information, of the greatest interest in recent years, would have been desirable, but the observations were not carried out uniformly.

Acute nephritis: The serious prognostic import of lengthy oliguria — anuria with rising N.P.N. values is well known and obvious. Prognostically more uncertain are cases of uremia where the diuresis is normal or fairly good or where diuresis has increased (recommenced) after earlier oliguria—anuria.

Volhard, 1935, as well as Goldring, 1941, point out the fact that anuria is rare in cases of acute nephritis. Murphy, Grill and Moxon, 1934, state that anuria had a fatal issue in all of their 14 cases, without further details or any definition of their conception of anuria.

E. Petrén found anuria in 4 out of 304 cases of scarlatina nephritides; 2 of these died having had anuria for 4 and 5 days respectively; the 2 survivors had anuria for 1 and 4 days respectively.

Rudebeck, 1946, studied the prognosis of nephritis in this clinic from 1910—1939. Thus our materials coincide for a certain period. R. concludes as follows: »In complete or almost complete anuria the prognosis is very grave. Among 16 cases of this kind, 12 terminated fatally and 2 went to chronic nephritis.» Thereby R. defines anuria as amounts of urine less than 200 ml per 24 hours over several days. Only one of his 16 cases recovered and showed no symptoms on later examination.

R. found the following concerning N.P.N. values: 13 cases out of 35 with an N.P.N. level of 100 mg % or more died; in most of these cases the diuresis was less than 200 ml. Of 17 cases with an N.P.N. level of 200 mg % or more, 8 died; of 5 cases with N.P.N. 300 mg % or more, 3 died; of those who survived N.P.N. values of 200 mg % or more, 5 were found to be normal on later examination and 3 cases were uncertain. Of those who survived an N.P.N. level of 300 mg % or more, one recovered completely whereas one showed chronic nephritis.

Volhard, 1935, observed a maximal N.P.N. level of 195 mg % in cases surviving uremia. Several authors among others Murphy, Grill and Moxon, 1934, and Murphy and Peters, 1942, emphasize the serious prognosis of uremia.

Fig. 1 gives the blood-N.P.N. levels and the diuresis during the patients' stay in hospital.

The amounts of urine checked in hospital must, as always, be regarded as a minimum value. On occurrence, discharge of urine observed together with faeces has been marked with a +. These values, like the information given in the following have been quoted from the case histories; the laboratory tests have been carried out at the routine clinic laboratory.

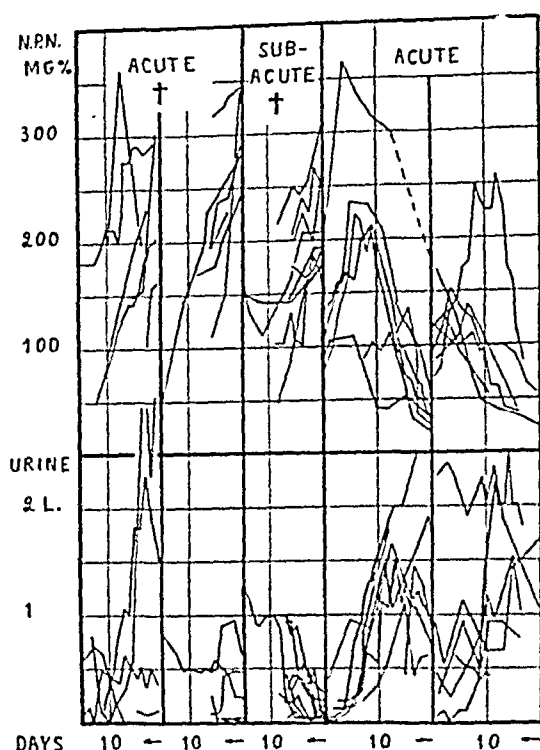


Fig. 1. The figure gives the blood-N.P.N. and the diuresis of dead acute—subacute and surviving acute nephritides during their time of observation in hospital. The last day of the patient's life is marked 0. For further particulars see text.

Though some anamnestic information may give an idea of the amounts of diuresis before the patients' admittance to hospital, this cannot, naturally, be regarded as reliable.

Table 3 gives particulars of the 20 cases, who died of acute—subacute nephritis. The autopsy diagnosis of cases 12—20 was subacute, of all the other cases acute nephritis. The table gives the age of the patient and the possible duration of the nephritis before death. The other information refers to the last 7 days of the patients' life, not counting the day of death; in 1/3 of the cases the time of observation was less than 1 week. The information comprises: 1) highest blood value, 2) highest

Table 3.

The table gives particulars of 20 cases, who died of acute—subacute (cases 12—20) nephritis. The table gives the age of the patient and probable duration of nephritis before death. Other information concerns: 1) highest value of blood-pressure; 2) upper and lower limit of pulse rate, omitting day of death. The information comprises: 1) highest value of blood-pressure; 2) upper and lower limit of pulse rate, blood-N.P.N., and specific gravity of urine, and finally 3) possible febrile condition. Thereby no records have been kept of possible rise of temperature during the last two days with accompanying increase of pulse rate.

No.	Age	Dura- tion, weeks	Blood- pressure	Pulse rate	Tempe- rature	N.P.N. in blood mg %	Urine, ml per day. — Day							Urine, specific gravity
							7	6	5	4	3	2	1	
1	32	2	90	70—80	—	118—205	5	5	0	15	0	0	0	—
2	54	2	160/65	80—120	febrile	188—226	0	0	25	0	0	0	0	—
3	13	2	125/80	70—70	—	322—342	—	—	—	40	0	0	0	—
4	59	4	120/75	80—100	—	111—340	—	250	50	50	50	40	20	—
5	37	1	150/95	90—100	febrile	103—163	—	—	125	90	70	80	100	1.010—1.012
6	18	2	120/80	90—130	*	190—286	—	70	500	650	500	400	225	1.020—1.013
7	34	4	100	70—80	—	223—290	620	500	420	520	320	500	360	1.011—1.010
8	59	3	160/90	100—80	—	170—225	450	300	300	150	350	350	450	1.015—1.010
9	59	5	185/100	90—90	febrile	227—277	500	450	500	800	850	900	600	1.013—1.008
10	43	2	140	90—100	*	360—200	1,000	1,000	1,800	1,800	2,300	1,900	1,500	1.010—1.009
11	37	2	135/80	80—100	*	148—300	560	1,300	2,700	4,900	1,800	3,050	3,050	1.011—1.018
12	45	2	150/105	80—100	—	49—180	—	300	110	170	75	35	17	—
13	42	7	140/90	60—90	—	200—275	100	100	20	100	30	0	30	1.013
14	22	2	135/85	70—80	—	100—182	900	550	500	325	175	50	50	1.010—1.013
15	61	30	130/80	70—90	—	150—167	400	250	200	150	125	40	55	—
16	56	4	130/80	70—70	—	235—310	550	250	300	400	300	500	80	1.029—1.007
17	15	7	125/90	70—50	—	210—210	400	450	250	45	40	42	105	1.011—1.007
18	18	2	190/115	100—120	—	143—190	—	—	450	550	450	45	150	1.015—1.009
19	63	2	140/70	110—140	febrile	196—270	300	450	450	350+	250	200	200	1.012—1.010
20	53	2	175/100	80—90	—	154—168	1,000	800	900	200+	400+	+	275	1.012—1.013

and lowest values of pulse rate, blood-N.P.N. and the specific gravity of the urine during the last week, and 3) febrile condition if any; possible rise of temperature during the last two days with accompanying quickening of the pulse rate has not been recorded.

Diuresis. In the following we report the amounts of urine 1) during the period of observation up to the death of the patient, and 2) until the last 4 days of the patient's life. That last-mentioned day has been gratuitously chosen as the last day on which it would have been possible to use dialytic treatment before irreversibly fatal damage had occurred.

(1) During the complete period of observation the amounts of urine have varied between total anuria and up to normal values, the latter having been observed even during the last week of some patients' life. Total anuria was found only in 3 cases out of 20. A maximum of 25 ml was found in one case over 7 days, in another over 11 days. 25—50 ml was found in 2 cases over 2 days, in 3 cases over 3 days, in 1 case over 4, and in 1 case over 5 days.

In $\frac{1}{2}$ of the cases diuresis has amounted to at least 100 ml/day, in $\frac{1}{3}$ 200 ml and in $\frac{1}{5}$ at least 300 ml/day. In three of the cases diuresis was small 15—10 days before death but increased during the last week; in one more case to more than $\frac{1}{2}$ liter and in the other two cases to several l. per day. In one more case oliguria was followed on the 6th day before death by passage of up to $\frac{1}{2}$ liter. In these cases the nephritis must have been improving but the renal function insufficient to remove the uremic intoxication in time.

(2) Table 3 shows, that the amounts of urine passed from the 7th to the 4th day (the day which has been gratuitously chosen as the last possible day for dialytic treatment) before the patients' death were as follows: In $\frac{2}{3}$ of the cases at least 100 ml/day, in $\frac{1}{2}$ at least 200 ml/day, in $\frac{1}{3}$ 300, in $\frac{1}{4}$ 400 and in $\frac{1}{5}$ at least 500 ml per 24 hours. In several cases the diuresis does not decrease during this space of time, but remains constant or even increases considerably. The specific gravity of the urine is practically invariably low in spite of oliguria. The increasing concentration of the blood-N.P.N. or its insignificant reduction despite good diuresis, signals, however, the seriousness of the prognosis.

Case no. 11 succumbed with nephritis during scarlatina. After temporary oliguria, diuresis recommenced. After an initial rise, the blood-N.P.N. level sank for a short time but in spite of the passage of large quantities of urine it began to rise again. At another hospital the patient was given fluid as well as bicarbonate. As uremia increased and the condition of the patient deteriorated, he was transferred to the clinic for possible dialytic treatment. On his arrival he was exsiccated. N.P.N. level 300 mg %, bicarbonate in serum 7.4 m. mol., blood-pressure about 60 mm Hg. He died after a couple of hours. Autopsy revealed acute nephritis and purulent bronchitis (table 1).

In this case acidosis—exsiccation will have been the essential cause of death; by supplying him with larger amounts of electrolytes and fluid in time, there might have been a chance of saving the patient.

Our material thus confirms that lengthy oliguria—anuria is a serious symptom. The amounts of urine can, however, be fairly good or even large so late that there is probably no chance of a favourable result with dialytic treatment. The above case seems to show the importance of control of fluid balance, and acid—base equilibrium in cases with large diuresis.

Uremia. Table 4 gives the degree and duration of the rise of the N.P.N. level in dead and surviving cases of nephritis. However, the information about the duration is often uncertain as uremia may often have appeared before admittance to a hospital. Apart from one exception among the surviving cases, the rise of the N.P.N. is — as expected — higher and more protracted in those who die from the disease. It is worthy of note, however, that 30 % of the dead never reached a level of 200 mg % N.P.N., 60 % did not reach 250 mg %, and 70 % did not reach 300 mg %.

We shall not discuss the relative toxicity of the retention products, only point to the fact that the blood-N.P.N. level offers good guidance for the estimation of the patient's condition, and further to the importance of the determination of the excretion of N.P.N. in the urine, which should be estimated against the background of the following quantitative relations:

One can allow oneself, as a rule of memory, to assume schematically that a rise of the blood-N.P.N. to 200 mg % corresponds to a total accumulation of about 100 g of N.P.N. in the body, 300 mg % corresponds to 150 g in all and so on. Thereby we assume an even distribution of the N.P.N. in 50 liters of body fluid. Against the background of these quantities and by determining the N.P.N. secretion in the urine one can get a rough idea of the patient's possibilities of becoming cleared through diuresis before the uremic intoxication has brought about a fatal issue.

Table 4.

The table gives the degree and duration of the rise of the blood N. P. N. in acute nephritides.

Nephritis	Non-protein nitrogen in blood, mg %						
	≤100	≤150	≤200	≤250	≤300	≤350	≤400
<i>A. Acute, surviving cases:</i>							
1. <i>Duration of uremia</i>							
1 day — Number of cases	—	1	—	1	—	—	—
2 days " " "	—	—	1	—	—	1	—
3 " " " "	—	1	—	—	—	—	—
4—6 " " " "	6	1	2	—	—	—	—
7—9 " " " "	1	1	1	—	—	—	—
10—12 " " " "	4	3	—	1	1	—	—
13—15 " " " "	1	1	1	—	—	—	—
more than 15 days " " "	2	—	—	—	—	—	—
Average days	9.6	7.3	6.7	6.5	—	—	—
Limits "	4—7	1—13	2—13	1—12	—	—	—
2. <i>Percentage of total number of cases</i>	100	57	45	14	—	—	—
<i>B. Acute—subacute, deaths:</i>							
1. <i>Duration of uremia</i>							
1 day — Number of cases	—	—	1	1	3	—	—
2 days " " "	2	2	1	3	—	—	—
3 " " " "	—	2	3	1	1	—	—
4—6 " " " "	7	6	4	3	2	—	—
7—9 " " " "	6	4	3	—	—	—	—
10—12 " " " "	2	4	2	—	—	—	—
13—15 " " " "	1	—	—	—	—	—	—
more than 15 days " " "	2	1	—	—	—	—	—
Average days	8.7	7.0	5.6	2.3	2.8	—	—
Limits "	2—28	2—17	3—10	1—6	1—6	—	—
2. <i>Percentage of total number of cases</i>	100	95	70	40	30	—	—

Thus it may be stated that a fairly good diuresis does not exclude the need for dialytic therapy in cases of acute nephritis with a high blood-N.P.N. level. On the other hand the well-known fact is confirmed, that patients can survive considerable oliguria for several days on condition that the N.P.N. level does not rise too much, fig. 1.

Oedema. As is well known, the risk of oedema of the lungs in acute nephritis is a serious danger. In table 3, oedema of the lungs has been recorded in cases no. 5, 8, 10, 14, 15, 18, and 19. Among these we find most of the cases who showed

high pulse rate and died at comparatively low N.P.N. levels. Here we have reason to assume that oedema can have been an essential cause of death. In cases no. 9 and 12 bronchopneumoniae can have contributed towards the fatal issue; case no. 12 died at an N.P.N. of 180 mg %. On the other hand uremia may of course have been the cause of pneumonia. Only one case, no. 4, had cramps.

The greatest risks to a patient suffering from acute renal failure are oedema and uremia.¹

The risks of oedema (i. e. fluid retention) in acute nephritis with oliguria are of great practical importance:

1) *In treating uremia by extracorporeal dialysis of the blood or peritoneal or intestinal irrigation the therapy itself must not expose a patient with acute nephritis to an additional supply of water with its accompanying risk of oedema.* This demand is satisfied in the construction of a dialyzer, described by one of us, A. 1947.

2) *In cases of acute nephritis with possibly fatal oedema rapid removal of fluid can be expected to save the situation.* In the modification of the above mentioned dialyzer described by one of us, A, 1947, it is possible to remove fluid from the blood by means of ultrafiltration as it passes through the apparatus.

3) *The dangerous fluid therapy is often used without weight control in cases of acute nephritis, to make diuresis recommence or increase. It can, however, involve fatal oedema; e.g. case no. 5, table 3 presumably died essentially from oedema of the lungs, as a result of an intense fluid therapy, carried through before her admittance to our clinic.* On the other hand the following case illustrates the value of thirst therapy to allay oedema-fluid retention; more cases are published in another connection.¹

A woman, 29 years old became acutely ill with pain in the region of the kidneys; oliguria, and dark urine. She was admitted to a surgical department with suspected renal calculus or hemoglobinuria after suspected hemolytic attack. She was encouraged to drink as much as possible; planned active fluid therapy was not carried through.

Because of increasing uremia she was transferred to this clinic for dialytic therapy if necessary.

¹ Cf. Allwall's publications in Nord. Med. (1948, 40, 2378) and the Lancet (in press) on the treatment of acute renal failure with anuria-oliguria where it is pointed out that a surplus of fluid is a fundamental premise for oedema (i. e. fluid retention) under these conditions and where the hazards of excessive electrolyte-fluid supply are emphasized.

Table 5.

The table shows the results of essential laboratory examinations in a case of acute nephritis, treated with hunger-thirst. For particulars, see text.

Day Nr	Body weight kgm	U r i n e				B l o o d	
		Volume ml per day	N.P.N. mg %	Chloride mg %	Specific gravity	Pressure	N.P.N. mg %
1	—	50	—	—	—	120/85	72
2	—	100	—	—	—	165/110	79
3	—	+	—	—	—	170/100	119
4	72	40	—	—	1.011	180/105	140
5	72	100+	—	—	1.011	175/100	146—143
6	71.8	100	175	267	1.013	170/95	174—151
7	70.8	200	175	215	1.015	150/90	170—178
8	69.3	425	455	274	1.014	165/100	185—250
9	68.3	950+	444	135	1.012	155/95	233—250
10	—	1,400	560	132	1.011	160/90	235—216
11	65.8	2,000	740	110	1.011	160/90	222—174
12	62.7	2,200	730	89	1.011	150/105	266—222
13	63.2	2,000	960	70	1.009	130/95	222—235
14	62.9	2,800	665	79	1.008	140/100	174—160
15	62.3	2,000	740	69	1.009	140/100	138—154
16	62.5	2,000+	400	85	1.007	135/100	114—108
17	61.5	1,800	400	83	1.006	130/80	69—69

Table 6.

The table shows the frequency of acute exacerbations (microscopic changes) in autopsy material of chronic nephritides.

Age, years	Nephritis chronica	Nephritis chronica cum exacerbatione acuta
11—20.....	2	0
21—30.....	6	3
31—40.....	1	6
41—50.....	6	2
51—60.....	10	1
61—70.....	6	5
71—80.....	1	0
Total	32	17

Patient had no apparent oedema. She was treated by thirst and hunger for 5—6 days, her weight was checked daily. Diuresis soon recommenced the patient rapidly losing 10 kg weight, her general condition improving simultaneously. Pain that had so far persisted subsided; blood-pressure went down to normal.

As an indication of the improvement in her renal functions the N.P.N. concentration in the urine increased to a maximum of 960 mg %; the blood-N.P.N. sank gradually.

In the following the results of certain other laboratory examinations taken in the beginning and, in brackets, at the end of her stay at the clinic are reported; Indican ++ (—), xanthoprotein 82 units (52), uric acid 8.0 (6.5) mg %, total base 135 (148) m mol., bicarbonate 23.5—18.0 (12.5—11.0) m mol., chlorides 294—270 (290) mg %, calcium 10.6 (10.1) mg %, potassium 18.0 (18.0) mg %, phosphorus 7.9 (3.1) mg %.

At the beginning of her stay in hospital both her kidneys were greatly enlarged, on re-examination 2 weeks later they showed normal size on the X-ray films.

It seems probable that the thirst-hunger treatment in this case saved a life and that a more intense fluid therapy at the onset of the disease would in all probability have proved fatal.

Summary — Acute Nephritis.

In this connection the main therapeutic problems in cases of acute nephritis are: uremia and oedema.

1. Pronounced oliguria—anuria during several days combined with a rising blood-N.P.N. level is a serious prognostic symptom that may indicate dialytic treatment.

2. The amounts of urine may in cases with a lethal course remain fairly good—normal to the very last; consequently too great a regard to the amount of diuresis may in this situation cause dangerous optimism. If the enhanced blood-N.P.N. remains unchanged in spite of good diuresis the situation should be judged as serious and dialytic treatment considered. The value of the determination of the N.P.N. excretion in the urine and the importance of certain quantitative views on the N.P.N. accumulation in the body, given above, are emphasized.

3. The degree of the rise of the blood-N.P.N. appears to offer good guidance, if no oedema of the lungs or other serious complication supervenes. The duration is certainly of great importance: the higher and the more protracted the rise of the N.P.N., the more serious the prognosis, because of the risk of irreparable damage, and the greater the indication for dialytic treatment.

4. The new possibilities of treating possibly fatal oedema with Allwall's »artificial kidney» are mentioned.

5. It is necessary to obtain a view as comprehensive as possible of the general condition etc. of the patient based on the results of

modern laboratory examinations *and on continual checking of the patient's weight*. If possible one should try to attain the desired therapeutic relief by earlier approved methods for the treatment of uremia; the value of hunger-thirst treatment (without Volhard's "Wasserstoss"!) for the combating of oedema may be emphasized. On the other hand necessary treatment through extracorporeal dialysis of the blood (or some other method with the same effect) or possible active removal of fluid from the body in order to allay possibly fatal oedema should if possible be carried out before the condition becomes irreversible.

The above attempts to give a firmer foundation for the estimation of the indications for dialytic therapy in the individual case until further experiences of these methods have been gained. Of course it is impossible to decide now whether this therapy would actually have improved the prognoses of the cases discussed above.

Kolff, 1946, sums up his indications for dialytic treatment of uremic patients with the artificial kidney as follows: »We propose to treat the patient with the artificial kidney as soon as the urea content of the blood rises over 350 mg per 100 cc (3.5 g per litre) and before that, if, moreover, the potassium content of the blood is higher than normal, or the alkali reserve is too low.»

Chronic Nephritis.

In the stadium of insufficiency of chronic nephritis, uremia often develops slowly, unlike the fairly speedy supervention in acute nephritis. The patients generally apply to the hospital only when a temporary deterioration makes the uremia more pronounced or in the final stage. Consequently the observations we report below only refer to a short space of the course of the disease.

Contributory causes of uremia or essential symptoms are often cardiac insufficiency with oliguria, in the isosthenuric patient, insufficient supply — loss through vomiting of fluid, with oliguria, acidosis, disturbances of the electrolyte balance and so on. Therefore a material of chronic nephritides would not be suitable for our purpose without a thorough analysis of each separate case with regard to among others the above mentioned factors, and steps taken to compensate the disturbances etc. In spite of the fact that this has not been possible in the present material it has seemed desirable to record the values of blood-N.P.N. and diuresis of our chronic nephritides who died of uremia in the final stage of their disease, fig. 2. Here we find higher and

more prolonged rises of the N.P.N. than in the acute cases. As expected, the diuresis decreases sub finem vitae, but as a rule not to such a degree as in the acute cases.

The indications for dialytic treatment of chronic nephritis will mainly be the same as those for the acute cases, reported above.

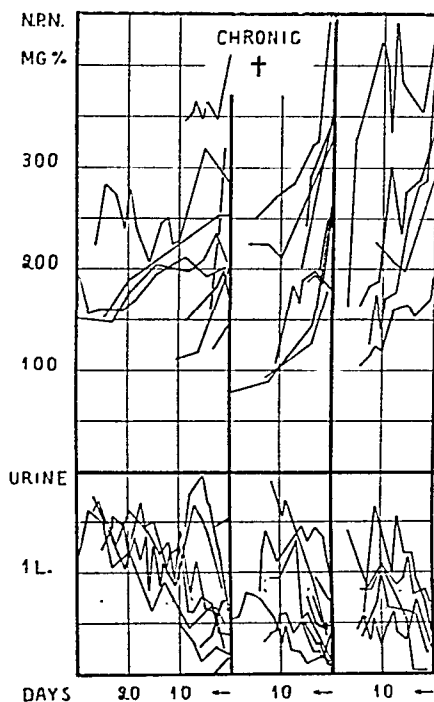


Fig. 2. The figure gives the blood-N.P.N. and the diuresis of dead chronic nephritides during their time of observation in hospital. The last day of the patient's life is marked 0. For further particulars see text.

However, we want emphasize the well-known value of the usual methods of allaying the above mentioned disturbances (fluid therapy to attain sufficient diuresis, allaying of electrolyte disturbances, heart drugs etc.) through which uremia can often be removed. Above this we should like to add the following:

1) As particularly suited for dialytic treatment, one may perhaps, like Kolff, 1946, regard those cases which have deteriorated in connection with an exacerbation of the nephritis.

Table 6 shows that, of our material of dead chronic nephritides, on autopsy about $\frac{1}{3}$ showed acute changes in addition to the chronic nephritis. On autopsy it is certainly difficult to decide what contribution the exacerbation has made towards death in

the individual case. It often seems impossible to diagnose the acute attack definitely in vivo on the basis of data available in the case history. The knowledge that acute exacerbations do occur in so many cases seems, however, to increase the indications for dialytic treatment of chronic nephritis.

2) According to the opinion stated by one of us, A. 1947, there would also, however, seem to be chances of good therapeutic results in cases of chronic nephritis, where a deterioration with uremia is caused by factors other than acute exacerbation. During a deterioration period with uremia, the renal function may soon be sufficient to balance the daily out-put of secretion products. As a great rise of the blood-N.P.N. level is, as mentioned earlier, equal to the accumulation of considerable quantities of N.P.N. (and other retention products) in the body, a renal function that only balances the retention products formed during the day cannot in such a situation remove the uremic intoxication. Dialytic treatment at the right moment can, at least in certain cases, be expected to break such a vicious circle and lengthen life and capacity for work of patients with chronic nephritis with uremia.

Summary — Chronic Nephritis.

The indications for dialytic treatment of chronic nephritis largely coincide with those of acute nephritis. However, special conditions are to be found in chronic nephritis. The importance of those disturbances of the circulation, fluid balance, acid—base balance etc. that secondarily cause uremia, and the possibilities of regulating them by other methods than dialytic therapy are emphasized. Certain views are given on the value of dialytic treatment in cases that cannot be helped by other methods, and on the importance of acute exacerbations of nephritis.

Kolff's, 1946, indications for dialytic treatment of uremia have already been quoted above.

Summary.

The earlier material of the clinic of acute and chronic nephritides with uremia and a blood-N.P.N. level above 100 mg % has been collected. On the basis of the result of the examination of the material the prognosis of the present cases are discussed

with regard to the indications for extracorporeal dialysis of the blood and, if necessary, removal of oedema with the aid of our »artificial kidney».

As to the results, the reader is referred to the summaries of the earlier sections: acute and chronic nephritis respectively.

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On the Artificial Kidney VII.

Clinical Experiences of Dialytic Treatment of Uremia.

By

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Haas, 1928, 1935 attempted treatment of uremic patients with an apparatus of similar construction to the one which Abel, Turner and Rowntree, 1913—1914, used in their experiments on animals. Kolff and Berk, 1944, and Kolff, 1946, were the first to use an apparatus of such capacity that the yield from the dialysis could be of human therapeutic value. We return to their results later.

In the following we report our experiences of dialysis with our first 12 patients. Some of the earliest results have been reported before (the authors, 1947, 1948).

All the patients were treated with a dialyzer of the first type, where the cellophane tubing is wrapped round a cylinder of wire netting and surrounded by a mantle of the same material. For methodological and technical details the reader is referred to earlier papers in this series.

As a rule both cylinders of the apparatus have been employed, consequently 10—11 m of cellophane tubing: dialytic area about 6 500 cm². In cases no. 2, 10, and 12 because of lack of tubing or some other reason only the big outer cylinder has been employed (6—6.5 m.: about 3,600 cm²); in cases no. 6 and 8 one cylinder was employed for most of the treatment.

The salt solution has as a rule had the composition described in paper III of this series. However the concentration of all the electrolytes has been reduced by 5 % in the treatment of the last 3 cases.

In the latest 2 cases citrate has been added to the saline solution in order to reduce the need for heparin. This problem has already been mentioned in an earlier paper, and will be discussed again later.

At first the haemorrhage from the operation wound, through which the cannulae were inserted in the arteria radialis and the vena mediana cubiti, caused certain losses of blood, which could, however, without inconvenience be replaced by small transfusions. In the last seven cases the blood vessels were prepared the day before commencement of dialytic treatment, as in the experiments on animals. There was practically no haemorrhage from the operation wounds due to the heparinization in these cases.

During dialytic treatment the arm, whose vessels are connected to the apparatus, is fixed in a splint. Earlier, when the patient could not be connected to the apparatus immediately after insertion of the cannulae, or when the treatment was interrupted for a time, we used to let the blood flow from the arteria radialis through a glass capillary back to the vein. However this arrangement required heparinization of the patient.

Later we found that it was sufficient to fill the rubber tube, one end of which is closed and the other end connected to the cannula in the blood vessel, with 1—5 per cent heparin solution; in this way we consequently have no arterio-venous anastomosis, and coagulation in the cannulae can be prevented for a long time without heparinization of the patient. Having found it possible to continue the treatment without interruption for about 24 hours or what time may be required to essentially allay the uremia, we have now ceased to work on this technical detail. We now regard it as better to remove the cannulae after a sufficiently lengthy, uninterrupted treatment, and to insert them again in another place when necessary.

Where not otherwise stated, the apparatus has been placed 30—40 cm below the patient. This difference of level is sufficient to prevent inflow of fluid to the blood passing through the cellophane tubing.

The following information is given *partly* by means of 2 tables containing essential data of the cases and *partly* by short surveys of diseased states and the patient's reaction to treatment, followed by a discussion of the most essential details. However we still regard it as too early for detailed analysis of individual cases and await further experiences. We refrain from a penetrating comparison with the experiences published by Kolff, 1944, 1946.

Casuistics.

(Tables 1—2).

The cases no. 1—3 have already been reported, the authors, 1947. During the year that has passed since the treatment of case no. 2, she has felt fit and has been capable of working although troubled by ulcera varicosa. At the last control examination urea clearance was 60 %; with thirst tests the urine could be concen-

Table 1.

Case No.	Age years	Sex	Body weight kg	N.P.N. in blood, mg%		N.P.N. yield g	Duration of dialysis hours	Total amount of salt-solution litres	Dialysis area cm ²	Heparin			Blood pressure		
				before	after					Initial doses mg	Total amount mg	mg per hour and kg	initial	final	lowest highest
1	49	M.	61.5	418	320	45	6	84	1250	1025	8	3.3	160/100	135/90	130/75
2	54	W.	94.0	235	182	18	8	68	800	650	34	3.0	175/100	180/100	175/95
3	44	W.	57.3	346	180	34	5	68	250	1125	15	0.7	140/90	145/100	140/100
4	57	W.	74.5	200	70	28	8	112	6500	1500	24	0.4	115/65	125/70	65/40
5	53	M.	67.0	222	57	70	20	244	6500	1125	25	1.1	120/70	120/80	110/60
6	38	M.	84.2	275	174	40	10	244	6500	675	13	0.6	140/100	175/105	105/55
7	57	M.	72.7	364	71	80	46	6500	300	600	16	0.7	185/95	170/100	165/100
8	36	W.	78.5	292	100	70	200	—	200	625	22	0.5	170/100	165/100	135/85
9	48	W.	52.0	148	83	64	90	3600	250	550	13	0.6	180/100	200/100	145/80
10	49	W.	55.6	320	133	13	112	3600	200	—	—	0.8	180/105	175/95	165/85
11	32	M.	58.7	333	160	55	90	6500	100	580	36	—	145/80	175/100	180/110
12	37	W.	200	160	63	11	134	3600	100	200	10	0.3	210/100	185/95	150/100
			266?	105	21	10	68	—	100	300	13	0.3	125/80	165/90	140/70
			180	100	48	11	112	6500	—	—	—	0.4	225/130	215/120	115/50
					?		90	—	—	—	—		130/75	—	130/60
														125/70	155/75

Note. Blood-pressure recordings are 1) the initial value at the beginning of dialysis, 2) the final value at the end of the dialysis, and 3) the lowest and the highest value that were determined between the initial and the final value.

trated to 1.024; proteinuria 0.3—0.6 ‰; on microscopic examination of sediment isolated red blood corpuscles and isolated cylinders; blood-pressure 130/80. The insertion of the cannulae had left small scars and the arteria radialis pulsated in a normal way about 2 cm above the scar on the wrist.

Case 4. A woman 57 years old, who 28 and 2—3 years ago had noticed temporary haematuria. Repeated infections of the urinary tracts during the last 9 years; at the onset of these disturbances cystic kidneys had been found on the roentgen films. During the year prior to admittance to hospital increasing fatigue, poor appetite, and loss of weight. Was unconscious one morning and had cramps, wherefore she was admitted to the clinic.

Patient presented the usual picture of serious chronic uremia with general disorders, uremia, acidosis, anemia etc. Urea cl. 7 ‰. In spite of fluid and alkali therapy etc. she did not improve during the following two weeks: Nonprotein nitrogen (N.P.N.) of the blood at first remained unchanged at 175—200 mg% then gradually rose. Nausea made it difficult for her to drink.

Dialytic treatment carried through without any complications except for a chill of short duration about 1 ½ hours after the entry of the donor blood into the patient's circulation. Continual moderate haemorrhage from the operation wounds. After about 12 hours' dialysis patient was considerably clearer and could speak distinctly for the first time for several days.

Post treatment course. Her temperature, subfebrile before the treatment, remained unchanged. Blood-N.P.N. gradually rose and after 4 days was about 100 mg% and after 10 days reached 125 mg%. Her fatigue soon decreased and her general condition improved, her appetite was rather good and she was practically free from any feeling of nausea. Patient could leave her bed and sit on a chair and do needlework for the larger part of the day.

During the last ten days of the patient's life temperature began to rise, tachycardia, increasing N.P.N., deteriorated general condition. Conforming with the wish of her relatives a second treatment had to be postponed until after patient's birth-day, when she was highly febrile and unconscious.

Renewed dialytic treatment. 45 days after the first. The patient's condition was little improved by the treatment.

Autopsy showed bilateral cystic kidneys, cystic liver, hypostatic bronchopneumoniae and oedema of the lungs.

This patient with chronic uremia caused by cystic kidneys was subjectively and objectively improved for one month after treatment. N.P.N. rose slowly and not until one month after treatment reached the same level as before the treatment. The last treatment,

six weeks after the first one, was applied so late that there were hardly any chance of a therapeutic result.

Case 5. A man, 53 years old with uremia of about $1\frac{1}{2}$ years' duration, who had earlier been admitted to the clinic and had been discharged with a blood-N.P.N. level of 150 mg%. He was re-admitted two weeks later because of diarrhoea. N.P.N. the first day was 148 mg%. Urea clearance 5 %. The diarrhoea continued and he began to feel sick and vomit, general condition deteriorated and N.P.N. rose above 200 mg%.

Dialytic treatment: At first general condition improved and patient could drink without feeling sick, but listlessness followed in connection with a gradual rise of temperature to 39.9° C. at the end of the dialytic treatment. Treatment was not interrupted in spite of the rise of temperature.

Post treatment course. The following morning the temperature was 36.8 and patient remained afebrile. On the fourth day N.P.N. had reached 100 mg% and later rose further and during the following month was between 150 and 200 mg% as before treatment. However, patient felt considerably better after the treatment, his nausea and diarrhoea disappeared and his appetite improved for a time. He could be looked after at home and was comparatively free from disturbances in spite of the fact that N.P.N. was about 200 mg%.

Half a year later he died from increasing uremia.

Autopsy showed cystic kidneys.

A temporary deterioration of chronic uremia caused by cystic kidneys was allayed through dialytic treatment and the general disturbances decreased in spite of the fact that N.P.N. soon rose to earlier level.

Case 6. A man, 38 years old, who 10 days before his admittance to the clinic had shown dark urine and increasing headache, sickness, haemorrhagic and slimy feces. Blood-N.P.N. began to increase after oliguria with amounts of urine less than 100 ml per diem, haematuria, proteinuria, etc. He showed obvious oedemas, was psychically clear but troubled by sickness and vomiting.

Dialytic treatment. No complications apart from a chill $1\frac{1}{2}$ hrs after the commencement. During treatment obvious subjective and objective improvement of general condition, sickness disappears. The oedema in the face possibly subsided somewhat.

Post treatment course. Patient felt well, was possibly euphoric. On catheterization two days after treatment, having had anuria, the yield was 25 ml strongly haemorrhagic urine with a small coagulum. The following two days the yield was 65 and 25 ml respectively of macroscopic haematuria.

On the fifth day after cessation of the dialytic treatment the blood-N.P.N. had risen to 178 mg%. In the evening the patient had a serious attack of oedema of the lungs, which was repeated during the following days. The diuresis was then practically nil. The N.P.N. increased and the patient's general condition was affected, tachycardia and rise of temperature.

Renewed dialytic treatment was applied 9 days after the first. 2—3 hrs later patient had another attack of lung oedema and died.

Autopsy showed acute—subacute glomerulonephritis. Sinistral cardiac hypertrophy and oedema of the lungs. The dissector called attention orally to the presence of unusually large haemorrhages of the kidneys and suggested that this might be connected with heparinization.

In a case of acute glomerulonephritis we managed to control the N.P.N. rather well with dialytic treatment; but we did not succeed in our attempts to reduce patient's oedemas by removing fluid from the body. Before the last treatment he had repeated attacks of lung oedema and died of a renewed attack after the cessation of the last treatment.

Case 7. A man, 57 years old with grave hypertonia for several years began to show symptoms of uremia. During the first days N.P.N. was 114—146—148 mg%. He was troubled by headache, nausea and asthma cardiale. On his own definite request dialytic treatment was applied.

Dialytic treatment was short. Moderate haemorrhage from the operation wounds; one ligature had slipped from its place. The loss of blood was compensated by a transfusion.

Post treatment course. The patient stayed at the clinic for two weeks. N.P.N. did not rise above 83 mg%. Sickness diminished, otherwise status quo.

Short dialytic treatment of a case of hypertonia essentialis with nephrosclerosis and a slight rise of the N.P.N. Certain subjective improvement.

Case 8. A woman, 36 years old, who for $\frac{1}{2}$ year had become increasingly tired, felt sick and out of breath; was admitted to the clinic comatose. Temperature rises to 39° C. and pulse rate to 110.

Dialytic treatment was applied during the second day of her stay at the clinic. She had then already shown symptoms of lung oedema. She was deeply unconscious so that the cannulae could be inserted without anaesthesia. After 9 hrs' dialysis a technical mishap occurred: the temperature regulator had not been functioning; the temperature of the saline solution was 48 degrees C. In connection with this rise

of temperature, which was probably of short duration, her blood-pressure sank to 110/80. The treatment was immediately interrupted and the blood-pressure rose again.

Some 6 hrs later patient died without having regained consciousness.

Autopsy showed hypoplastic left kidney, cyst in the right one and chronic glomerulonephritis. Sinistral cardiac hypertrophy. Purulent bronchitis and oedema of the lungs. No sign of damage by the above mentioned temperature rise in the apparatus.

(*Note:* Since that time we control the temperature of the salt solution every quarter of an hour and record the degree.)

A moribund patient was treated with dialysis. Before treatment she had had attacks of lung oedema. Patient did not recover consciousness. She died a few hours after the cessation of treatment; in connection with exitus symptoms of oedema of the lungs.

Case 9. A woman, 49 years old. Proteinuria in connection with partus 14 years ago. Since then subjective good health, no urine examination. Since one month previously increasing fatigue, since one week beginning of oedema and dark urine, had grown pale. After her admittance to the clinic febrile, tachycardia, diarrhoea, and commencement of vomiting. The amount of urine sank to 100 ml per diem and less. Her general condition deteriorated more and more. 9 days before death, confluent foci of miliar type were found on X-ray examination of the lungs. Penicillin treatment.

A delivery of cellophane tubing from America having been delayed, dialytic treatment could not be applied until late in the course of the disease. Patient had deteriorated more and more, and was comatose when the treatment began; eight hours earlier, oedema of the lungs had supervened.

The apparatus was placed 50 cm lower than the patient for the treatment. A slight chill, beginning 2 hrs after the commencement, subsided after 20 minutes. During the treatment patient appeared better, the symptoms of oedema of the lungs decreased, she woke from her somnolence and asked for coffee which she consumed with relish. Thereafter she slept quietly for a couple of hours. The coagulation time had increased to more than 100 minutes. The pulse rate was as high as before, 120—130, and the rate of respiration was 25—29 per minute. As the blood-pressure had dropped to 110 and the haemoglobin from initial 40 % to 27 % a transfusion was given. Thereafter everything looked well until patient began to have difficulty in breathing. Blood-pressure was 140/70. One hour later patient died of increasing lung oedema.

Autopsy showed acute-subacute glomerulonephritis and signs of a slight, older attack of nephritis. Haemorrhagic diathesis with multiple subserous haemorrhages; haemorrhage of the lungs and lethal intestinal haemorrhages, signs of toxic effects in the vessels of the kidneys, lungs, and spleen. Pronounced anaemia. Oedema of the lungs. Transudate in right and left pleura. Old apical scar in the right lung.

Microscopic examination showed i. a.: »Tubuli contain ample hyaline cylinders and are in some places packed with red corpuscles. This effusion of blood constitutes a prominent feature of the picture». — »The lungs show extensive profusion of blood in alveoli. Several heart-failure cells are to be seen and here and there coal-pigments. Some of the bigger vessels show polypous wall thrombs, covered with endothelium and built of fibrin. — A cut through the arteria pulmonalis on the site of the autochthonous thromb reveals a partly organized thromb with small isolated blood vessels growing in from the wall of the vessel.»

This patient, suffering from acute—subacute nephritis with uremic intoxication in an advanced stage and oedema of the lungs died during treatment from haemorrhage complications, which are regarded as due to a haemorrhagic diathesis as a consequence of uremia and heparinization. The case seems to illustrate: a) the risk of haemorrhage during dialytic treatment on account of the heparinization and b) the probably increasing risk of haemorrhage the more lingering the uremia. Also in this case patient had oedema of the lungs before the application of the treatment; this oedema possibly decreases in connection with treatment and increases in connection with exitus.

Case 10. A woman, 49 years old. In connection with partus 27 and 25 years ago infection of the urinary tracts. Had had infected urine later also. For about $\frac{1}{2}$ year tired, pale, small appetite. At her admittance blood-N.P.N. was 180, acidosis, anemia, etc. Infected urine. Urea cl. 8 %. During 2—3 weeks progressing deterioration, continual sickness, vomits, increasing fatigue. N.P.N. level rose to 200 mg%.

Dialytic treatment was applied without complications. In the beginning still sickness and vomiting; these symptoms ceased gradually and general condition improved. The first dialytic treatment was interrupted in the evening. During the night arterio-venous anastomosis and continued heparinization; treatment was continued the following day.

Post treatment course. Not until the 10th day had the blood-N.P.N. level risen from 100 to 150 mg%, on the 15th day to about 200. The value remained constant between 150 and 200 mg%. Urea cl., determined on different days, was 8 and 5 % respectively. All the time during the first month patient felt rather well, and definitely better after the dialytic treatment. Gradually sickness recommenced. After a short stay at home, she returned to the clinic much deteriorated and 3 months after the dialytic treatment she died from uremia and oedema of the lungs.

Autopsy showed pyelonephritic nephrocirrhosis, hypertonic heart, signs of failing cardiac function with stasis organs. Hydrothorax. Lung oedema.

A chronic nephritis with chronic uremia improved subjectively for about one month after treatment; nausea and vomiting disappeared for the same space of time. About two weeks after the treatment the blood-N.P.N. level was the same as before the treatment. Patient died of uremia and lung oedema three months later.

Case 11. A man, 32 years old with an anamnesis of tiredness and hypertonic troubles for more than a year was admitted with high blood-pressure, anemia, pronounced changes of the retina, etc. High pulse rate and rising temperature. After a severe attack of cramp patient became unconscious. No improvement after withdrawing cerebrospinal fluid and dehydrating therapy.

Temperature rose to 39.6° C. On request of his relatives dialytic treatment was attempted.

Dialytic treatment. The apparatus was placed 70 cm lower than the patient. During the course of treatment patient became clearer, was sometimes restless, appeared to recognize his relatives.

During the following 24 hours patient lay unconscious with lung oedema towards the end. Exitus.

Autopsy showed chronic nephritis with moderate cirrhosis of the kidneys. Bilateral subdural haematomas, which had, according to the dissector, come about before the treatment. Oedema of the lungs.

The estimation of the possible effect of the treatment in this case of chronic nephritis was complicated by subdural haematoma, which had arisen before the dialytic treatment. Attempts at removing fluid through the apparatus seem to have had little or no effect.

Case 12. A woman, 37 years old, who after partus showed oliguria—
anuria and rising N.P.N. values. The last day her sight became impaired, normal retinas. Severe oedema of the lungs occurred the day after her admittance to the clinic, which could only with difficulty be mitigated through inhalation of oxygen under excess of pressure. As patient was moribund, dialytic treatment was applied in order to remove fluid in that way if possible. The apparatus was placed 70 cm lower than the patient; at first 5 %, later 7.5 % glucose was added to the saline solution. The blood sugar value the whole time was about 500 mg%. In spite of these steps as little as at the most 100—150 ml fluid per hour seem to have been removed from the patient.

Before the treatment her sight was so reduced, that patient could neither read nor tell the time by the clock. She spontaneously declared that her sight had improved considerably during treatment and she could tell the time without difficulty. Possibly some improvement of general condition. She could do without the gas mask for short intervals. However the lung oedema increased in strength. Patient died some three hrs after the cessation of treatment.

It was not possible to allay the oedema in this case either — acute nephritis with lung oedema — by steps in connection with dialytic treatment.

Discussion of the Material.

The casuistics and tables 1 and 2 show mainly the following:

Material. 12 cases of uremia have been treated. In two cases, nos. 3 and 10, the treatment lasted with intervals more than 48 hrs. In two cases, nos. 4 and 6, a renewed treatment took place after 45 and 8 days respectively. Consequently 14 (16) treatments have been carried out in these 12 cases.

The cause of uremia was in one case blocking of the ureters due to cancer, in one case hypertonia with nephrosclerosis, in two cases cystic kidneys, in 4 cases acute—subacute nephritis and in 4 cases chronic nephritis; of the last mentioned group one case furthermore showed hypoplasia of one of the kidneys and a cyst in the other.

The main reason for dialytic treatment was in 9 cases uremia. In two cases the treatment must essentially be regarded as an attempt to allay life threatening oedema (as well as the uremia). In one case psychological considerations caused us to apply a short treatment at an N.P.N. as low as 125 mg%.

From the clinical data given above it appears, that $\frac{1}{2}$ of the cases were treated for the first time so late, that death from uremia or oedema of the lungs was impending (cases nos. 1, 3, 8, 9, 11, and 12); the condition was similar on the application of the second treatment in the cases no. 6 and 10. In case no. 3 the lethal course was known in advance on account of cancer.

When treatment was started for the first time in the 12 cases and when treatment was repeated in the two cases, the blood-N.P.N. was in 12 cases, at least 200 mg%, in 8 cases, at least 250 mg%, in 5 cases at least 300 mg%. The uremia has thus as a rule been grave. In this connection we shall not discuss the significance of other symptoms in the uremic picture.

The treatment has thus in several cases, for different reasons been carried out under such conditions, that a lasting therapeutic result could not be expected. The presented material can thus only show, that the method can have therapeutic value, but not to what extent good therapeutic results are to be expected. However it gives many valuable experiences for the future work.

Table 2.

Case No.	N.P.N. mg%		Uric acid mg%		Xantoprotein (units)		Indican		Protein %		Total base (m.mol.)		Bicarbonate (m.mol.)	
	before	after	before	after	before	after	before	after	before	after	before	after	before	after
1	418	320	—	8.0	—	—	—	—	—	—	157	169	20.0	21.4
2	235	182	10.0	7.3	70	62	++	++	7.2	6.0	150	148	18.0	—
3	346	180	13.3	7.3	140	80	++	++	7.7	6.3	157	157	12.1	19.1
4	200	70	9.3	4.6	120	70	++	++	6.5	5.0	161	158	15.7	20.3
	222	57	6.4	3.7	95	65	++	++	8.1	6.7	158	174	16.2	19.7
5	275	174	—	—	116	96	++	++	8.1	6.9	169	171	—	—
	234	71	7.0	2.3	—	—	++	++	7.3	6.7	155	155	—	—
6	364	100	9.4	8.1	45	38	++	++	5.3	4.8	162	149	—	—
7	292	118	13.3	8.3	—	—	++	++	6.2	5.5	143	152	21.4	20.5
8	148	83	—	—	—	—	(++)	0	5.0	4.8	135	139	23.2	24.0
9	320	133	—	—	178	90	++	++	6.9	6.0	139	—	25.1	—
10	333	160	13.3	6.3	90	78	++	++	6.7	5.7	133	144	16.7	—
	200	160	8.9	7.3	25	15	++	++	7.9	6.9	143	146	22.3	18.0
11	160	105	—	—	30	15	++	0	—	5.1	148	147	19.0	18.5
	266?	140	16.0	10.0	100	90	++	++	6.5	5.0	145	150	18.0	19.0
12	180	100	16.0	13.0	50	46	++	(++)	6.2	6.2	145	150	15.5	20.1

Blood-N.P.N. The effect of the dialytic treatment on the blood-N.P.N. naturally depends on the concentration of N.P.N. in the blood, the length of the dialysis, and the amount of blood passing through the apparatus and the size of the dialytic area. As earlier mentioned, the dialytic area has in most of the cases been about 6,500 cm², in some cases only about 3,600. In the majority of the cases the rate of flow through the apparatus will have been about 8—10 litres/hour. The amount of N.P.N. eliminated corresponds as a rule with the amount which can be calculated from the change of the N.P.N. of the blood during dialysis and the assumption of an even distribution of the N.P.N. in the total amount of fluid of the body.

Simultaneously with the decrease of the blood-N.P.N., its content of uric acid, xanthoprotein, and indican decreases, Table 2.

«*Total protein*» in plasma — with regard to our laboratory resources determined according to van Slyke's copper-sulphate method — practically without exception decreases to some degree. To this we may, however, add the critical comment, that with this method the specific gravity of the drop of plasma is determined, its value is reduced, when urea and other substances leave the blood during the dialysis. We will in the future examine possible changes of the serum protein with other methods.

Electrolytes in the blood. The value of the total base as a rule has not changed if normal or — if too low — changed in the normal direction. We shall not enlarge here on the problem as to whether one ought to aim at normalizing the total base in cases with changes in connection with different causes of uremia.

Among other examinations we have omitted the results of the determinations of calcium, potassium, phosphorus, and chlorides, which have as a rule been carried out before and after dialytic treatment, as these have not shown any changes of interest.

The bicarbonate value does not change during dialysis if normal or becomes normalized, if it has been previously too low.

Duration of treatment. It is of great interest to note, that it has been possible to extend the treatment over 20 hrs or more without noticeable inconvenience to the patient; in this way as much as 150—200 litres of blood comes in indirect contact with about 200—250 litres salt solution.

Blood-pressure. In this connection the behaviour of blood-pressure is of great interest. Because of the construction of the appa-

ratus, with constant amounts of blood decided in advance, and independent of variations of pressure in the cellophane tubing, the flow of blood to the apparatus is always equal to the inflow back to the patient. As is shown by table 1 practically without exception the blood-pressure shows only insignificant fluctuations, even in cases of lengthy dialysis.

Thus we have not seen the serious conditions of shock which Kolff noted in several of his patients. As has been stated in another connection these shocks can be due to the construction of Kolff's apparatus: The patient can lose too much blood into the long cellophane tubing the content of which can not be controlled.

The heparin dosage is an essential point. From fear of coagulation in the beginning we used large doses of heparin, like Kolff. Thus in the first case we used an initial dose of 1,250 mg. During the 8 hours of dialysis we gave another 375 mg. In our latest cases we have reduced the initial dose to 100 mg and not more than 200—300 mg heparin in all during 10—12 hrs' dialysis.

From Kolff's work, 1946, the following reports about the heparin dosage may be quoted: during 6—7 hours' dialysis the following amounts were given in different cases: (3,400, 2,100 mg heparin), 8—9 hrs (2,750, 2,300, 2,200, 2,150, 1,280, 1,025 mg), 10—12 hrs (1,200, 1,075 mg), and 13—14 hrs (3,350, 3,200, 1,820, 1,200).

In table 1 we have also recorded the heparin dosage in mg per hour and kg body-weight: the value has gradually been reduced from 3.3 mg to 0.3—0.4 mg. After our experiences with case 9 we have especially aimed at reducing the heparin dosage.

In another connection we have already stated that the construction of our dialyzer is calculated to reduce the risk of coagulation of the blood. The blood need not, as in Kolff's apparatus pass rotating couplings, a rotating cylinder, and Beck tube pump; the length of the cellophane tubing in our apparatus is only $\frac{1}{4}$ of that in Kolff's apparatus, and the blood is pressed in a thin layer through the tubing. In Kolff's apparatus there seem to be possibilities for the blood to stagnate in an expanded tubing, whereby the risk of coagulation increases.

The favourable effect of dialytic treatment on the general condition of the intoxicated patients appears from the casuistics. In an amazingly short time the psychological bluntness, the nausea and vomiting diminish or even disappear altogether while the treatment is still in progress. This improvement may last even when the blood-

N.P.N. has risen again to comparatively high levels. In this connection we will only mention that substances other than N.P.N. are removed by dialysis and stress the well known fact that N.P.N. may not even be the essential factor in uremic intoxication.

In acute nephritis the patient may be threatened by the uremic intoxication and the tendency to oedema.

(a) The possibility of efficiently removing the accumulation of N.P.N. by dialytic treatment has already been shown.

(b) Our apparatus offers the important practical advantage of adjusted hydrostatic pressure, preventing the inflow of water from the saline solution to the blood passing through the cellophane tubing. It should thus save the patient from additional risk of oedema in connection with dialytic treatment.

The above mentioned possibility does not exist in Kolff's apparatus as has earlier been mentioned.

By lowering the dialyser to 70 cm below the patient — the greatest difference of height that has been practically possible — and by adding up to 7.5 % glucose to the solution we have not managed to remove enough fluid from the patient that this step can be supposed to have had practical value for patients with oedema (lung oedema etc.).

That Kolff should really be able to make fluid flow from blood to the saline solution, by 2—3 % glucose in the solution, in therapeutically sufficient amounts, does not appear to have been proved, as information of weight i. a. is lacking.

(c) A modified construction of the apparatus is necessary to attain this therapeutically important aim. Such a construction is to be described in a future paper, it has already been preliminarily reported, A. 1947.

Possible complications of dialytic treatment will be briefly mentioned.

It appears from the casuistics that oedema of the lungs has in several cases been noted before the treatment and reappeared after it. In the cases, where lung oedema did not appear before but during or after the treatment, the treatment has been applied so late in the course of the disease, that the oedema would in all probability have appeared even if dialytic treatment had not been applied.

The possibly increased strain on the heart by the flow of blood (8—10 l. per hour) from the arteria radialis (through the appa-

ratus) to the vena mediana cubiti has not proved to cause any signs of cardiac insufficiency.

Temporary chills have in some cases appeared at the beginning of the treatment, about $1\frac{1}{2}$ —2 hours after connection of the apparatus to the blood vessels (entry into the patient's circulation of the donor blood). These chills do not seem to have noticeably affected the condition of the patients.

Haemorrhage. We have earlier commented on the risk of haemorrhage, involved by heparinization in cases of uremia. Thereby we have also pointed out the importance of keeping the heparin dosage low and other steps to reduce the need of heparin. In one case with far advanced uremic intoxication and haemorrhagic diathesis the heparinization was followed by fatal haemorrhages. This risk appears to increase with the severity and the prolongation of the uremia.

In one case small amounts of haemorrhagic urine were discharged after the treatment. On the other hand we have seen cases, where earlier existing microscopic hematuria did not increase in connection with the heparinization. In cases, where passage of urine has existed before the treatment, discharge has often occurred during the treatment.

Contrary to Kolff, 1946, we have no hemolysis problem.

Finally we give a short summary of Kolff's therapeutic results.

In his dissertation, 1946, Kolff reports 15 cases. We quote the following from the report of a lecture, *Lancet*, 1946 (II. p. 726): "Among the first fifteen cases treated, one survived, a patient with anuria following chemotherapy. It could not be said, however, that in this case recovery was due to dialysis, as the usual therapy alone might have been sufficient. Ten more patients had since been treated, with four survivals. A woman of 67 with acute cholecystitis and glomerulonephritis, and complete anuria, who before treatment with the kidney was fully expected to die, was one of the recoveries. The other three were a man with prostatic enlargement, stones in the bladder, and in one ureter, and chronic cystopyelonephritis, and a girl of 13 and a man of 54, both with acute glomerulonephritis. The girl was comatose, with oedema, anuria, and bronchopneumonia; dialysis reduced her blood-urea from 364 to 140 mg per 100 ml, and diuresis set in during dialysis. The man was similarly very ill, with almost complete anuria, hiccup, mild oedema, and pulmonary congestion; his blood-urea was reduced from 324 to 172 mg per 100 ml; in this case the diuresis did not appear until 5 days later, and use of the kidney may well have prevented fatal toxæmia.

Kolff, like us, often seems to have applied dialytic treatment at such a late stage, that his results can not be regarded as significant of the therapeutic possibilities of the method. For reasons given above we refrain from a critical comparison in this connection between Kolff's results and ours.

Summary.

We report our experiences of dialytic treatment of 12 uremic patients. The therapeutic value of the method for the elimination of uremic retention products is shown. The need for a technique, which above this allows the removal of fluid from patients with serious oedema is pointed out. Steps taken to prevent complications are discussed.

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